CONTRIBUTIONS TO STRESS RESPONSE RESEARCH: TELOMERE LENGTH AND TRANSGENERATIONAL ASPECTS IN FORMER INDENTURED CHILD LABORERS

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Andreas Lorenz Küffer

of Bern, Switzerland

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on the recommendation of the Doctoral Committee:

Prof. Dr. Dr. Andreas Maercker (main advisor),

Prof. Dr. Dr. Ruth Lanius and Prof. Dr. Birgit Kleim

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“The greatest glory in living lies not in never falling, but in rising every time we fall.”

Nelson Mandela

(Long Walk to Freedom, 1994)

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Abstract

Traumatic and adverse events have a deep impact on affected individuals – not only short-term after the experiences, but also on the long-term. It potentially alters their stress perception and regulation, and subsequently even affects behavioral and cognitive levels. It is assumed to take its toll on biological and familial components of a traumatized individual.

The overall goal of this cumulative PhD project was to investigate the biological and transgenerational consequences of childhood adverse experiences. Therefore, a sample of Swiss former indentured child laborers and a sample of matched controls were investigated. Paper 1 of this thesis is a narrative review article that investigates and summarizes the assumption of transgenerational inheritance of stress- and trauma related telomere length (TL) erosion. The aim of Paper 2 was to assess if TL was significantly shorter in individuals with a history of childhood adverse events (CAE) and post-traumatic stress disorder (PTSD). Paper 3 explored potential transgenerational consequences of CAE. Therefore, the data of offspring from Paper 2 were investigated, mainly focusing on adverse events in their own childhood, their psychopathology, sense of coherence and optimism/pessimism. Furthermore, parental rearing behavior was taken into account as a potential mechanism for transgenerational trauma transmission.

Conclusions from Paper 1 suggest that traumatic adverse experiences are a form of "acquired" stress-that might be passed down to subsequent generations in form of shorter TL and therefore cause physiological disadvantages in subsequent generations. Nevertheless, despite a growing body of literature that says otherwise, as Paper 2 indicates, in this sample there was no indication that CAE or PTSD is negatively associated with shorter TL. Lastly, in Paper 3 the transgenerational data suggest that offspring from parents with CAE experience more CAE themselves, but show no

increased psychopathology compared to the offspring of healthy controls. Nevertheless, offspring from traumatized individuals indicated an impaired sense of coherence.

The here presented findings are discussed in the context of current state of research. Furthermore, limitations and shortcomings of the here presented research are addressed, and further implications for future research will be offered.

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1 Introduction

Trauma and adverse life experiences imply enormous psychological suffering and burden for people who experienced them., They sometimes affect an individual for the rest of their life (Hiskey, Luckie, Davies, & Brewin, 2008) and are associated with other severe mental illnesses (Mueser, Rosenberg, Goodman, & Trumbetta, 2002). Involuntarily, they affect all aspects of the victims' lifetime development, from psychological to biological aspects, including their social and familial interactions. This is particularly the case if the traumatic or adverse experience centers around social and familial events in the first place (Kellermann, 2001).

The affected individuals' children are therefore specifically vulnerable to the long-term consequences of their parents' suffering. This PhD thesis contributes body of research that is aimed at biological and transgenerational aspects of stress related disorders in a sample of former indentured Swiss child laborers ('Verdingkinder'). The thesis treats current biological models for trauma and stress related disorders and extends the existing body of literature for the former indentured child laborers with latest empirical findings from telomere length (TL) research. Furthermore, it extends the research to the consequences of former Swiss child labor for the subsequent generation, investigating the potential psychological health consequences for children of the above-mentioned Verdingkinder.

Three scientific articles form the core of this cumulative PhD project. One of these articles is a narrative review article that has been published in a peer-reviewed journal, and two empirical study articles were ready for submission at the time when this thesis was defended. These three articles are fully provided at the end of the thesis in sections 5 to 6. Before providing a summary of these three papers in section 3.2 to

3.4, section 2 provides a theoretical summary of the current background to the outlined project aims. Specifically, section 2.1 provides a short description of the specific sample of former Swiss child laborers and a short summary of the most important findings from this sample so far. Section 2.2 provides the theoretical background for biological trauma sequelae, while section 2.3 focuses on current theoretical background for trauma transmission. The thesis is finalized with section 4, which provides an overall discussion of the whole project, including limitations, clinical and research implications for future work and concluding remarks on the project.

2 Background

2.1 Former Child Labor in Switzerland

The following section will provide a short overview over the cultural phenomenon known as Swiss child labor. The first sub-section 2.1.1 summarizes shortly the historic background of the given subject, while sub-section 2.1.2 provides some background information on the samples that participated in Paper 2 and Paper 3.

2.1.1 Historic Context

In Switzerland between the 19th and later 20th century, orphans were unsystematically but frequently displaced into foster care families or orphanages by local authorities. That also extended to half-orphans, children from single parent families, and children who were socially unwanted or from low socio-economic backgrounds. These foster families were most typically families that were short of workforce, such as farmers or small craft enterprises (Leuenberger & Seglias, 2008). This was widely considered to be a social welfare intervention, established to provide familial care and education for children who otherwise would have been considered to be deprived of such benefits. The children, at times as young as just a few months up to late puberty (Maercker, Krammer, & Simmen-Janevska, 2014), were expected to work and contribute with hard manual labor in those foster families. Often, many of these families were also very poor and did not have the necessary means to adequately provide for these children. Working days were long, often started around 4 to 5 a.m. in the morning and lasted until late in the evening, leaving only little to no time for school or other activities. Besides the threat of food and sleep deficiency as well as harsh physical endeavors, many of these foster children were also exposed to psychological and social adversities. In some harsh and extreme cases, families intentionally did not treat the orphans as family and

deliberately isolated them by denying them joining family lunch or dinner, disallowing them to enter the living room or making them sleep with the cattle in the barn. Additionally, being a so-called "Verdingkind" was also a social stigma that led to low acceptance amongst peers and in the community in general. Therefore, many of these children suffered great emotional, physical – and in some cases – even sexual abuse.

To date, there is no official documentation of this practice. There is also no exact estimate of how many children were treated as badly as described above, since in other cases, the integration worked well. It is, therefore, not possible to provide exact numbers of people that were affected by the devastating negative aspects of this practice. Extrapolated from an estimate that this was done until the late 70s, a current couple of thousand former "Verdingkinder" still live to date, currently ranging from 50 to over 90 years of age (Leuenberger & Seglias, 2008).

2.2 Adverse Childhood Experiences and Early Life Trauma

An important assumption in developmental psychopathology states that adverse life events, maltreatment and trauma may be more consequential for children than for adults because of the complex interaction between trauma, psychological and neuro-biological development (De Bellis, 2001). Furthermore, in the case of interpersonal stressors, not only does the trauma (e.g. physical, emotional or sexual abuse) or the adverse experiences *per se* inflict pain and suffering and therefore cause psychopathological consequences, but they also disrupt the interpersonal relationships of a traumatized child (De Bellis, 2001).

According to the DSM-5, psychological trauma involves an exposure to death or serious injury as well as sexual violence (American Psychiatric Association, 2013), all of

which may be experienced directly (exempt death), witnessed, learned about or repeatedly experienced by subjects in detailed and extreme manner. Physical and sexual abuse may therefore be considered particularly potent traumatic stressors (Cloitre et al., 2009), but they do not remain the only ones. Other forms of childhood adverse events (CAE), such as physical and/or emotional neglect, emotional abuse, separation from parents or psychiatrically burdened parents may also result in long-lasting developmental consequences (Bernstein et al., 2003; Cloitre et al., 2009). According to Bernstein et al. (2003), abuse includes serious physical maltreatment that potentially leads to injury, any sexual behavior between the child and an older person, as well as emotional abuse, manifesting itself through a child's threatened sense of worth or well-being. Neglect refers to the failure of addressing a child's emotional and/or physical basic needs.

Prevalence and frequencies of childhood trauma and CAE vary between different populations and countries, are greatly context-specific (Wendt, Abrahão, Kluwe-schiavon, Sanvicente-vieira, & Luz, 2016) and hard to estimate due to methodological biases (e.g. mostly self-reported measures). In a representative sample of 6'787 Swiss adolescent students, roughly 56% reported at least one traumatic event during their lifetime (Landolt, Schnyder, Maier, Schoenbucher, & Mohler-kuo, 2013), and PTSD rates were estimated on 4.2%. In a German sample of over 3021 adolescents from 14 to 24 years of age, the lifetime prevalence for traumatic events was between 17.7% (female participants) and 26.0% (male participants) (Perkonigg, Kessler, Storz, & Wittchen, 2000). PTSD prevalence with 1.0% for males and 2.2% for females was substantially lower. Given that the two samples were relatively similar with respect to their cohort and cultural context, differences were probably mainly due to methodological differences (i.e. different assessment and classification of traumatic events). Schenk et

al. (2016) reanalyzed the data of the above mentioned 6'787 adolescent Swiss students with respect to the above-mentioned abuse and neglect types, and found that 39.5% of all participants reported any type of maltreatment, while 22.3% reported physical abuse by the caregiver, 26.5% reported emotional abuse, 6.4% indicated that they were neglected, 4.2% reported custodial interference, and 2.8% indicated sexual assaults by adults (Landolt, Schick, & Scho, 2016).

CAE and childhood trauma, too, have negative long-term consequences for adult psychological well-being later in life. For instance, a 10 year longitudinal study on over 420 subjects who entered the last data collection wave, abused individuals tended to be more depressed and anxious than participants without history of abuse (Ernst, Angst, & Monika, 1993). CAE are associated with an increased risk of developing substance abuse disorders (Edalati & Krank, 2015) and other psychological disorders, such as eating disorders, personality disorders, schizophrenia and psychotic disorders (Passmann Carr, Severi Marins, Sringel, Lemgruber, & Juruena, 2013). Furthermore, evidence from longitudinal studies suggests that children who suffered traumatic events are at higher risk of developing chronic PTSD when they happened to be exposed to further traumatic events during their childhood (Copeland, Keeler, Angold, & Costello, 2007).

2.3 Biological Trauma Sequelae

Adverse and traumatic experiences are a dominant and heavy psychological burden for those who experienced them and often affect individuals long after the event took place (Brent & Silverstein, 2013; Springer, Sheridan, Kuo, & Carnes, 2003; Umberson, Williams, Powers, Liu, & Needham, 2005). A series of densely connected and linked biological systems are affected and consequentially affect an individual's physical,

emotional, cognitive and behavioral regulation (De Bellis, 2001). Subsequently, if caused during childhood, these effects from maltreatment also cause biological adverse consequences and worsen physical and mental health later in life (Danese & McEwen, 2012). Danese and McEwen (2012) explain the association between early adverse psychological experiences and later biological consequences with the allostatic load (AL) model (McEwen & Stellar, 1993).

2.3.1 Allostatic Load Model and Traumatic Stress

The term "stress" is used in a wide variety of contexts, and summarizes a broad phenotype that represents an organism's total response to environmental demands or aversive experiences in general. Often, stress leads to pathological conditions, such as PTSD, and even suffering from a psychological disorder causes substantial amounts of stress. PTSD might be the most prominent and best example for a stress-related disorder, since its diagnostic criteria demand an enormous amount of endured stress, and suffering from PTSD itself induces stress (e.g. intrusions, hyper arousal; WHO, 1993). Stress is often used to cover a variety of negative environmental conditions influencing a biological system. It reaches from work-related stress to the burden of being a caregiver for seriously ill individuals (Kiecolt-Glaser et al., 2011), from experiencing childhood maltreatment (Shalev, 2012) to more severe forms of stress, such as being a rape-victim (Malan, Hemmings, Kidd, Martin, & Seedat, 2011) or having a childhood trauma (O'Donovan, Epel, et al., 2011). All above mentioned examples (and many more) may – even though not necessarily – induce a sufficient amount of stress and, as a result, cause psychopathology. One recurrent debate among researchers concerns the definition of stress in humans (McEwen, 2000) and whether it is primarily

to be considered an external response that can be measured by physiologic changes, such as cardiovascular symptoms (elevated blood pressure or heart rate responses (Dedert, Calhoun, Watkins, Sherwood, & Beckham, 2010), excessive transpiration or endocrinological reactions (e.g. altered corticotropin-releasing hormone responses in PTSD patients)(Bremner et al., 2003; McEwen & Stellar, 1993), or whether it represents more of an internal interpretation of – or reaction to – a stressor. What is most likely is a combination of both. It has consistently been demonstrated that individuals with stress and related disorders experience impaired physical and mental functioning, more workdays lost, increased impairment at work, and a high use of healthcare services. What is more, recent research in animal models and humans even shows transgenerational consequences of stress (Entringer et al., 2011, 2013; Field, Muong, & Sochanvimean, 2013; Yehuda et al., 2005).

Although stress *per se* is neutral and can be positive when the person feels stimulated and self-sufficient, more often than not, stress is perceived as negative when an individual is subjected to a severe or even traumatic amount, and it has consistently been demonstrated that individuals with stress and related disorders experience impaired physical, mental, and emotional functioning. Figure 1 by McEwen, (2000) describes the pathways of how environmental stressors and adverse life events are perceived differently by each individual. Stress perception induces behavioral responses and, thus, subsequently affects the biological or physiological stress response directly (and indirectly via said behavioral responses). Furthermore, stress perception is affected by individual differences that are influenced by genetic predispositions as well as individual experiences, such as CAE (McEwen, 2000, 2003). The cumulative load of the physiological stress response is referred to as allostatic load (AL), while allostasis is the adaption to the AL.

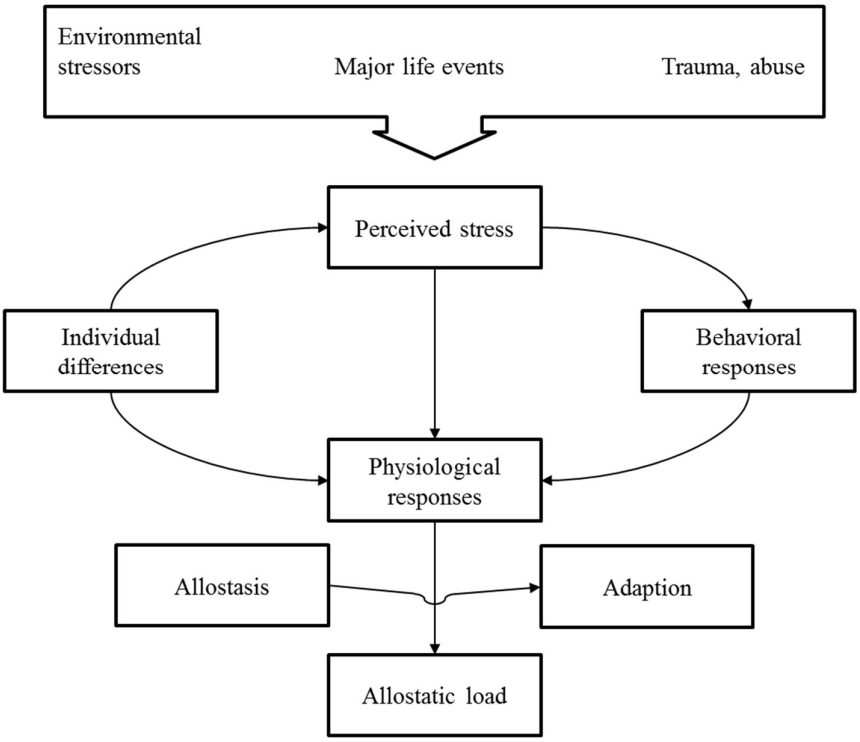

Figure 1. *Development of allostatic load through stress appraisal.*

Note. Adapted from " Allostasis and Allostatic Load: Implications for Neuropsychopharmacology" by B. S. McEwen, 2000, Neuropsychopharmacology, p. 114.

The AL model by McEwen and Stellar (1993) represents a potential paradigm of the effects of chronic psychological stress and how this affects an organism's functionality. According to the AL model, effects of stress might act on three different levels. First, primary effects take place on a molecular level. Cellular activities, such as gene-expression, receptors, enzymes, etc. are altered and affect the system on a very basic manner. Secondary effects take place on sub-clinical levels that affect physiological parameters (e.g. insulin, glucose, cholesterol, systolic and diastolic blood pressure, etc.), eventually leading to a range of stress-related diseases. The third stage

involves psychological deregulation and the development of diseases. Following this model, already small imbalances might set off severe consequences in the long run (Juster, McEwen, & Lupien, 2010). In the context of the AL model, biomarkers play a fundamental role, given that they either potentially cause or identify disease early on-set.

A number of working groups have confirmed the association between psychological stress and the biological marker of telomere length (Epel et al., 2004; Kananen et al., 2010; Kiecolt-Glaser et al., 2011; Malan et al., 2011; Tyrka et al., 2010), and findings suggest that TL might be a potential biomarker for sever psychological and traumatic stress.

2.3.2 TL as a Marker for Biological Stress

Telomeres are part of the DNA-strand, but do not contain specific genetic information to express RNA. They form a protective molecular structure, located at the ends of the human chromosome pairs, as well as at the ends of the other eukaryotes. Telomeres were fist described by Blackburn and Gall in the Journal of Molecular Biology (Blackburn & Gall, 1978). They are made of nucleotide sequences, organized in tandem arrays. The specific sequence varies between species and in humans the telomeric sequence is 5'-TTAGGG-3'. These telomeric tandem repeats form long strands up to in several hundred to thousand basepairs, depending on differences between species and between individuals. Telomeres are involved in maintaining genomic stability and regulating cellular proliferation (O'Sullivan & Karlseder, 2010). Telomeres have been shown to inhibit chromosomes to form end-to-end fusions by preventing the cell from identifying telomeres as DNA-double strand breaks (Jain & Cooper, 2010; O'Sullivan & Karlseder, 2010).

Due to mitotic division in somatic cells, telomeres progressively shorten with each mitotic division because of the inability of DNA polymerase to fully replicate the 3'-end of the DNA strand. Therefore each cell replication cycle causes a small fragment of telomeric DNA strand to loose, due to the nature of the replication process itself. This inability of copying the last nucleic acids results in a loss of about 50 basepairs of telomere sequences per cell doubling, what is known as the end-replication problem, which is partly held responsible for biological aging (Cech & Lingner, 2008; Harley, 1991; M. Z. Levy, Allsopp, Futcher, Greider, & Harley, 1992). If it were not for the telomeric structures, cellular proliferation would damage essential genetic information, and once the telomeres were shorten down to a critical length, the cell is endangered to suffer from the same fate. Hence, telomeres act as the "mitotic clocks" of our body, allowing a mitotic cell only to replicate itself for a certain number of times until its telomeres shorten down to this critical length and therefore lead to cellular senescence (Campisi, 1997). This was experimentally demonstrated in a longitudinal study conducted on transgenic mice, where the rate of increase in the percentage of short telomeres was predictive of the mice' life expectancy (Vera, Bernardes de Jesus, Foronda, Flores, & Blasco, 2012), and where telomerase-deficient cells with single short telomeres caused an earlier onset of senescence (Abdallah et al., 2010).

Telomerase is a ribonuclearprotein that counteracts TL shortening by a specialized reverse transcriptase, adding TTAGGG-oligonucleotides to the 3'-end of the DNA-strand. Telomerase is not active in all mitotic cells and is particularly important in cells that depend on replication of vast numbers (e.g. sperm cells, stem cells or activated lymphocytes). It is also believed to play an important role in immortalizing tumor cells by allowing them to intently proliferate without substantial TL loss. Exempt in sperm cells and tumors – where telomerase actually can elongate telomeres – telomerase can

only slow down TL erosion without preventing it completely (Gomez et al., 2012; Smogorzewska & de Lange, 2004).

TL erosion due to proliferation of mitotic cells is one important, though most probably not the only mechanism causing shortened TL. For instance, chronic inflammation may promote telomere shortening in part by increasing levels of oxidative stress, although this has only been suggested by animal models so far (Tilstra et al., 2012). Oxidative stress occurs when the enzymatic and non-enzymatic antioxidants fail to fully neutralize reactive oxygen species (Monaghan, Metcalfe, & Torres, 2009), chemically unstable molecules that tend to damage DNA by binding to nearby molecules (Fiskum, Murphy, & Beal, 1999; Monaghan et al., 2009).

2.3.3 PTSD, Childhood Trauma and TL

As indicated above, TL has been proposed as one of the mechanisms of life stress effects on physical health (Kendall-Tackett, 2009). A considerable number of studies – including experimental animal models (Herborn et al., 2014; Kotrschal, Ilmonen, & Penn, 2007) – have found significant relationships between stress, traumatic stress (and its consequences), such as PTSD and TL (Lei Zhang et al., 2014). O'Donovan et al. (2011) assessed 43 patients with chronic PTSD and 47 control subjects and found a significant association between PTSD diagnosis and TL shortening (O'Donovan, Epel, et al., 2011). Savolainen studied a sample of 1,486 adults (mean age of 61.5 years) at the time of tissue collection (Savolainen et al., 2014). They assessed separation from parents in childhood in a sample of 215 participants, as well as self-reported physically and emotionally traumatic experiences throughout the lifespan. Even though they did

not find a significant association between leukocyte TL (LTL) and either early life parental separation or emotional and physical trauma across the full sample, they found shorter LTL in the participants who were separated from their parents during childhood and who also reported traumatic experiences. A study of a sample of 3,000 subjects, PTSD alone was associated with shorter LTL (Ladwig et al., 2013). And in a study on 64 female rape victims, a marginal association was found between TL and PTSD in the 9 participants who developed a PTSD three months after the rape (Malan et al., 2011). Overall, these studies consistently indicate that PTSD status is associated with shorter TL.

Further studies have linked CAE with TL. In 2014, for example, the prospective longitudinal Dunedin Study conducted on 1037 subjects 11 years of age showed that persistent internalizing disorders in men – but not in women – predicted LTL at 27 years later in a dose response manner (i.e. the more years they reported to have suffered, the shorter were their LTL) (Shalev et al., 2014). In another cross-sectional study by Drury and colleagues including 80 children (age 5-15 years) exposed to family violence, the authors found that the number of adverse life events was associated with shorter buccal cell TL (BTL) (Drury et al., 2014).

As evidence for the potential causal role of CAE in shortening telomeres, some studies have reported a faster rate of decline in TL in children experiencing adversity. In one prospective longitudinal study of 236 children, Shalev and colleagues found that children with more exposure to violence showed significantly more BTL erosion from age 5 to 10 years compared to children with less violence exposure (Shalev et al., 2012). Similarly, Drury and colleagues published a longitudinal study on 136 children living in institutions where buccal swabs were collected between 6 and 30 months of age, and compared with BTL from 54 months of age (Drury et al., 2012). In their results, percent

time spent in the institution was significantly and negatively associated with BTL and remained so, even after controlling for potential cofounders like gender, ethnicity, low birth weight and age at telomere collection.

Overall, these studies consistently indicate that both CAE and PTSD status are associated with shorter TL. However, we do not know if CAE and PTSD status exert an influence on BTL over the entire lifespan into old age.

2.4 Transgenerational Transmission of Trauma

Like described above, psychological trauma has an enormous impact on an individual's psychological well-being. Besides often discussed consequences for psychological functioning or wellbeing of affected individuals, it also affects people in the close social and familial environment. Subsequent generations are generally at higher risk for trauma transmission, since they are both exposed to interpersonal and social trauma sequelae as well as to biological trauma sequelae.

Despite the argument that there is no transgenerational transmission of trauma (Sagi-Schwartz et al., 2003; Sagi-Schwartz, van IJzendoorn, & Bakermans-Kranenburg, 2008), evidence has also been found continuously for transgenerational trauma. In a study by Daud and colleagues, in which the authors investigated the effect of parentally experienced trauma (at least one parent was tortured for one month in Iraq or Lebanon) on their offspring, the authors found that their children, aged between six and 17 years, suffered from depressive and post-traumatic stress symptoms, somatization and behavioral disorders (Daud et al., 2005). In another study that has been conducted with Cambodian students, aged between 16 to 18 years, it was perceived that parental trauma symptomatology due to surviving the Khmer Rouge genocide was associated

with offspring's anxiety and depression (Field et al., 2011). While no significant association between maternal PTSD and offspring psychopathology was found in children of Rwanda mothers who survived the genocide of 1994 in Rwanda, maternal childhood violence exposure was associated with offspring's anxiety, depression, and behavioral disorders (Roth et al., 2014). Finally, in a recent study on adult children of holocaust survivors, it could have been shown that they had a lower sense of coherence when compared to the general population, suggesting lower resources to cope with one's challenges and adversities (Fossion et al., 2014). Together, existing data derived from transgenerational research suggest that paternally experienced adversity exert a meaningful impact on the next generation, with consequences for the offspring reaching from somatic to psychopathological symptoms.

Bowers and Yehuda (2015) describe several mechanisms of how parental stress might affect offspring. In line with the AL model (McEwen & Stellar, 1993) the stress can be biologically transmitted via the germline or *in utero* exposure to maternal stress (biological pathways of Figure 2). Pre- and perinatally experienced stress of mothers, for instance, has been shown to be meaningfully related to their offspring's cortisol levels (Yehuda et al., 2005), or LTL (Entringer et al., 2013).

Albeit many of these studies focus on maternal pre- and perinatal stress-transmission, it should be noted that paternal pre- and perinatal stress may also affect offspring development (Bowers & Yehuda, 2015). Although there is only one evident biological pathways of paternal stress transmission (i.e. via sperm), paternal stress might indirectly affect offspring well-being through paternal care.

2.4.1 Pathways of Psychological Transmission

Even after over decades of ongoing debates, the field of psychological, intergenerational trauma transmission is still a very active field of research. Throughout their lifetime children can be exposed to their parents' trauma by various means. For instance, parents can serve as a model for their offspring. If the parents' behavior in response to environmental inputs is affected by the trauma or severe stress exposure, children learn to react to this environment in a similar way (Bowers & Yehuda, 2015). Further theories involve psychodynamic theories such as projection and identification, as well as systemic factors, such as the atmosphere and communication within the family, or its functionality (Dekel and Goldblatt, 2008). Psychodynamic transmission (i. e. projection and identification) states that the traumatized parent fails to contain their negative emotions. As a consequence, negative emotions like aggression, shame and guilt are projected onto the offspring, who in turn starts to identify with these emotions. Dekel and Goldblatt (2008) further state that, as a result of this, the child may eventually fail to establish a separate self and develop psychopathological symptoms that replicate the parents' suffering. Atmosphere and communication within the family center more on systemic aspects of the family. The authors state that parents with PTSD, for instance, may have difficulties in regulating proximity and distance to the traumatic event (Dekel & Goldblatt, 2008). This inevitably affects the family atmosphere and the communication patterns of the parent-child dyads.

While these theories and factors may indeed be relevant in the transmission of parental adversity, they are difficult to be tested empirically. Parent-child interaction, i.e. parental bonding and rearing behavior, might be an adequate approach to approximate these factors (see psychological pathways of Figure 2), as parenting style can be easily assessed. Also, parenting style has repeatedly been linked to psychological

health of the offspring, though predominantly in samples with no particular focus on experienced adversity or trauma by the parents. In these studies, parental bonding and rearing behavior were associated with depression and anxiety (Lima et al., 2010; Rapee, 1997), schizophrenia (Skagerlind et al., 1996), personality disorders (Giakoumaki et al., 2013), and eating disorders (Tetley et al., 2014) in the offspring. In a Spanish sample with over one hundred female hospital patients, it was found that parental coldness, detachment, rejection and parental overprotection was related to self-reported childhood abuse and neglect (Hernandez et al., 2013). In a series of studies around the research group of Field and colleagues, it was found that the association between parental trauma and offspring psychopathology was mediated by parenting style i.e. parents' role reversing and maternal overprotection (Field et al., 2011). In 2013, the authors extended these findings by showing that parenting style mediated maternal PTSD symptomatology to offspring anxiety and depression (Field et al., 2013).

In summary, parenting rearing behavior might be a relevant candidate mechanism for the transgenerational transmission of early childhood adversities.

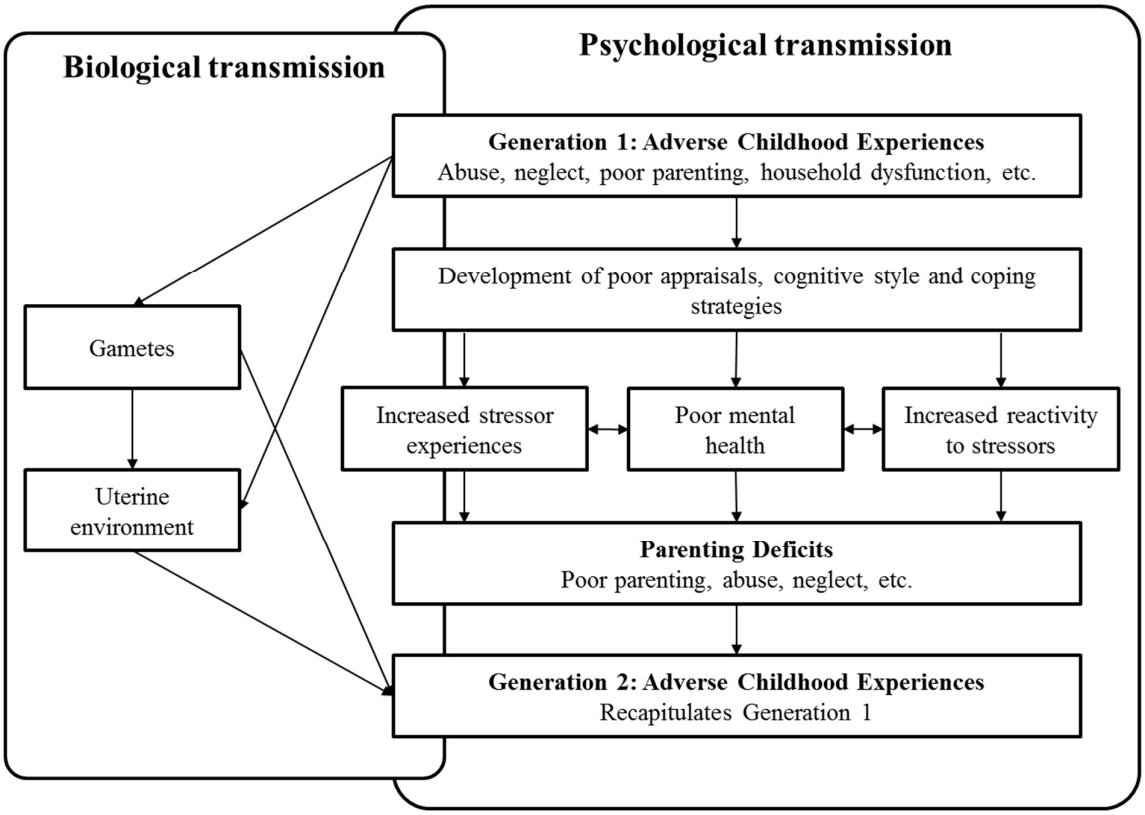

Figure 2. *Schematic representation of biological and psychological stress transmission*

Note. Stressful parental experiences have indirect effects on offspring psychological health via parenting and everyday interaction. Biologically, parental stress can be transmitted genetically via gametes (ante- and prenatally) or via the uterine environment (pre- and perinatally). Albeit biological and psychological pathways are described separately, both pathways are not independent from each other, and influence each other mutually. Psychosocial transmission adapted from "Intergenerational Trauma: Convergence of Multiple Processes among First Nations peoples in Canada" by A. Bombay, K. Matheson and H. Anisman, 2009, Journal of Aboriginal Health, p. 16 and biological transmission adapted from "Intergenerational Transmission of Stress in Humans" by M. Bowers and R. Yehuda, 2015, Neuropsychopharmacology, p. 234.

3 The Present Thesis

The following section will offer a short description of the aim of this dissertation and a short summary of the three publications. Each study will be described briefly, and the key contents of the papers will be highlighted. The empirical studies will be summarized in the four subsections background and objectives, methods, results, and the discussion.

3.1 The Study Samples

The here presented study samples are sub-samples from a larger project that originated in the year 2010. Psychometric data were originally collected between 2010 and 2012 for a longitudinal study on cognitive long-term consequences of childhood abuse. (Burri, Maercker, Krammer, & Simmen-Janevska, 2013). A number of scientific studies have so far been published (e.g. Kuhlman, Maercker, Bachem, Simmen, & Burri, 2013; Maercker, Hilpert, & Burri, 2015; Maercker et al., 2014; Simmen-Janevska, Horn, Krammer, & Maercker, 2014). The original sample consisted of 141 former Swiss indentured child laborers. Participants were all Swiss-German speaking, reported at least one traumatic event in their life, were at least 60 years of age and had at least one period of indentured child labor.

The project was later followed up with psychobiological aspects that included voluntarily donation of buccal epithelial cells. A sub-sample of 67 participants provided biological data in form of buccal cell swaps. In order to investigate transgenerational aspects of Swiss child labor, (Paper 3), the remaining participants again were asked to provide contact information of their children. Of the remaining 67 participants, 16 parents provided information of 22 children that were willing to participate in this study.

3.2 Aims

The aims of the dissertation centered on the project „Verding- und Heimkinder im Alter“. The original goal of this project was to investigate the long-term consequences of childhood maltreatment and its impact on adult and elderly development. The dissertation mainly focused on the transgenerational aspects of these adverse experiences.

The following research questions were primarily addressed:

Paper 1: What is the current status of empirical studies on the transgenerational findings for stress related disorders with respect to telomere research?

- How does psychological stress affect telomere erosion?
- What is the current state of research on transgenerational telomere research?
- Are there studies that support the assumption that traumata affect subsequent generations TL?

Paper 2: Do CAE affect BTL in a currently elderly sample of Swiss former child laborers compared to an elderly sample of control subject?

- Is current PTSD symptomatology associated with BTL?
- Are CAE associated with BTL?

Paper 3: Are there psychological transgenerational consequences of Swiss child labor in a sample of subsequent offspring form the above-mentioned samples?

- Do children from former child laborers report more psychopathology compared to children from controls?

- Do children from former child laborers report less psychological health indicators than children from control children?

3.3 Summary Paper One: A Transgenerational Approach to Telomere Biology in Stress Response Research

3.3.1 Background and Objectives

The first paper is a narrative review article that investigated and summarized the assumption of transgenerational inheritance of stress and trauma related TL erosion. The article focused mostly on the discussion of transgenerational aspects of TL maintenance with respect to childhood maltreatment, CAE, trauma and PTSD. By providing some theoretical background on how psychological stress might affect telomere biology, the article offered a hypothesis on how severe psychological stress might extend to further generations.

3.3.2 Transgenerational TL research

The subject of transgenerational aspects of TL biology is a relatively small and new research filed. A number of studies have pointed out that exposure to toxins during fetal and early postnatal development lead to increase adult onset disease (Bale et al., 2010; Barker, 2007; Godfrey, Gluckman, & Hanson, 2010; Jirtle & Skinner, 2007), but mediating biological mechanisms remain largely unknown. Two studies by Entringer et al. (2011, 2013), investigated the association between LTL and prenatal stress in mother-child dyads in a sample of healthy subjects. The authors found that prenatal and perinatal maternal stress exposure was linked to shorter LTL in newborns.

A different line of research suggested that the paternal germ line might play an important role for different reasons. Since sperm cells remain mitotic and proliferate throughout a healthy males lifetime, sperm TL increase, rather than decrease with age (Allsopp et al., 1992). The positive association between chronological age and sperm TL seems to have a direct impact on offspring TL thought the germ line. As presented in Table 1, a number of studies successfully showed that paternal age of conception and – subsequently – birth were associated with offspring TL.

3.3.3 Transgenerational Stress Response Susceptibility

Accumulating evidence indicates that stress response disorders, as for example PTSD, have a genetic component (Afifi, Asmundson, Taylor, & Jang, 2010; Stein, Jang, Taylor, Vernon, & Livesley, 2002; True et al., 1993). This indicates that stress response disorders might be a salient risk factor for the development of stress response disorders in subsequent generations. For instance, in offspring of a sample of 100 Holocaust survivors and offspring of 44 demographically comparable control subjects, the PTSD prevalence was significantly higher amongst children of Holocaust survivors (Yehuda, Schmeidler, Wainberg, Binder-Brynes, & Duvdevani, 1998). The same group extended their findings in other studies, indicating that trauma exposure and PTSD in mothers were not only associated with stress response disorders in their children, but also in with higher cortisol levels (Yehuda, Halligan, & Bierer, 2002).

Together with the findings on the influence of maternal stress on offspring TL (Entringer et al., 2011, 2013; Gapp et al., 2014), new findings from animal models that indicate that maternal stress might further be passed down with cortisol (Haussmann, Longenecker, Marchetto, Juliano, & Bowden, 2012), the hypothalamic–pituitary–adrenal

axis might suggest a link between the four factors of cellular metabolism, inheritance, telomere biology and transgenerational consequences of PTSD as well as other stress related disorders.

3.3.4 Discussion

The outlined findings in the presented review suggest that TL might be an important factor in transgenerational stress response research. Firstly, TL is a highly heritable endo-phenotype that implies the importance of transgenerational studies. Secondly, stress response disorders require a high amount of stress in order to develop. What is more, however, is that this stress also seems to affect TL erosion. Lastly, stress response disorders such as PTSD not only follow stressful events, but they also cause life-stress in patients. This stress is likely to contribute to TL erosion and maintenance.

The reviewed research suggests that this form of "acquired" stress-related TL erosion might also be passed down to subsequent generations and therefore causes physiological disadvantages in said generations. Nevertheless, to date, it remains unclear if this is the case and if potential compensating aspects like paternal age of conception might compensate for this effects. In addition, even though some studies have pointed out that prenatal and perinatal maternal stress exposure was associated with offspring TL, it remains an ongoing field of research if antenatal stress moderates germ-line transmission of TL.

3.4 Summary Paper Two: A Failed Attempt for Buccal Telomere Length Analysis in Old Age

3.4.1 Background and Objectives

Paper 2 is a cross-sectional group comparison of BTL in a sample of indentured child laborers and matched healthy controls. In previous studies, childhood maltreatment was repetitively associated with either shorter TL in children (Shalev et al., 2012) or later in life (Drury et al., 2012; Shalev et al., 2014). Separation from the biological parents after birth was also associated with shorter TL (Savolainen et al., 2014). Furthermore, studies have indicated that besides childhood maltreatment, PTSD is associated with shorter TL in adult populations (O'Donovan, Epel, et al., 2011) as well as in old age (Ladwig et al., 2013). For this study, a subsample of 62 participants from the original 'Verdingkinder' sample (Burri et al., 2013) was compared to 58 matched healthy controls. The main goal was to assess whether BTL was significantly shorter in the former child laborer sample with full or partial positive PTSD screening, compared to former child laborer without PTSD, as well as the control group. It was further assessed if childhood trauma exposure was negatively associated with BTL.

3.4.2 Methods

A total of 127 participants from both groups participated in this study, out of which 120 participants were analyzed (data of seven participants were excluded due to failed quantitative polymerase chain reaction (qPCR) approaches). The remaining samples consisted of 62 indentured Swiss child laborers (age: M = 76.19, SD = 6.18; 43.55% female) and 58 matched healthy controls (age: M = 71.85, SD = 5.97; 44.11% female). Potentially traumatic events were assessed with a short version of the Childhood

Trauma Questionnaire (CTQ; Bernstein et al., 2003) and PTSD screenings were conducted with the short screening scale for PTSD (SSS; Maercker & Pielmaier, 2010). In order to extract BTL, a qPCR (Cawthon, 2002) was performed on the donated buccal epithelial cells. The main analyses were controlled for age, gender, depression symptomatology, self-evaluated financial status and self-evaluated physical and mental functioning.

3.4.3 Results

In contrast to the hypothesis, former indentured child laborers screening positive for partial or full PTSD symptomatology showed longer BTL compared to healthy controls. These results remained significant even controlling for the covariates. The differences between the control group and indentured child laborers PTSD negative status, as well as the difference between indentured child laborers with PTSD positive screenings compared to those without PTSD status were both not significant. Also in contrast to the hypothesis, marginally positive association between higher CTQ scores and longer age adjusted BTL could be observed.

3.4.4 Discussion

In summary, this study failed to replicate the previously reported (Kiecolt-Glasera & Glaser, 2011; Ladwig et al., 2013; Savolainen et al., 2014) association between current PTSD symptomatology and/or childhood trauma exposure. The results of these analyses even pointed in the other direction, indicating that childhood trauma exposure and PTSD status in the old age was positively associated with BTL.

The results of this study possibly suffered from methodological shortcomings like a batch or selection effects. The results must therefore be interpreted with caution. On the other side, the results might also point towards a resilient-survivor-phenotype amongst the investigated participants, indicating that the elderly former indentured child laborers with higher exposure of childhood trauma and PTSD positive status from this study were a specific selection of resilient individuals.

3.5 Summary Paper Three: Psychological Aspects of Transgenerational Stress Transmission

3.5.1 Background and Objectives

Traumatic experiences may not only have a direct impact on the people who suffer from the trauma, but also on subsequent generations (Asok et al., 2013; Daud et al., 2005; Dekel and Goldblatt, 2008; Field et al., 2013, 2011). One plausible mechanism for transgenerational stress submission might be parental rearing behavior. Parental rearing behavior was associated with offspring psychopathology in studies that did not mainly focus on adversity and trauma (e.g., Giakoumaki et al., 2013; Lima, Mello, & Mari, 2010; Rapee, 1997; Tetley, Moghaddam, Dawson, & Rennoldson, 2014).

Paper 3 is a study that analyzed whether children from former indentured child laborers were themselves more often exposed to adversities and trauma in childhood. Furthermore, it was expected that children from former child laborers experienced dysfunctional parenting styles more frequently, which in turn affected the psychological health of these offspring.

3.5.2 Method

Parental and filial data of two groups were analyzed for this study. In the parental generation, a subsample of the previously introduced former indentured child laborer of 16 individuals and a subsample of 19 controls (also introduced in Paper 2) was analyzed. The filial generation consisted of 22 children from the indentured child laborer families (*M* age = 52.91, *SD* = 5.90; 68.18% female) and 29 children of the control group families (*M* age = 44.55, *SD* = 7.71; 68.97% female). For the filial sample, childhood trauma exposure was assessed with the CTQ (Bernstein et al., 2003) and parental rearing behavior was assessed with the Questionnaire of Recalled Parental Rearing Behavior (QRPRB, Schumacher et al., 1999). In order to assess psychopathology, the Brief Symptom Inventory (BSI, Derogatis and Spencer, 1993) was used, as well as the Revised Life Orientation Test (LOT-R, Scheier et al., 1994) and the Sense of Coherence Revised (SOC-R, Bachem and Maercker, in press) to assess a functional spectrum of psychological health. All statistical analyses were conducted with a Bayesian approach for statistical inference testing.

3.5.3 Results

There were no differences in general psychopathology between the two filial samples but some evidence that the children from former indentured child laborers themselves reported more physical childhood abuse as well as less expressed emotionality from their parents. Further, there was relative evidence that children from control families described themselves with a better sense of coherence, in particular better self-reflection. Bayesian analyses of covariance revealed no evident interaction between family type and emotionality or punishment, but indicated that lower family

emotionality was associated with higher psychopathology and higher pessimism. Independent of family emotionality or punishment, children of former child laborers had lower sense of coherence.

3.5.4 Discussion

This is a study with relatively small samples although in a two-generation control-group design. Even though childhood adversity, trauma, and parenting style data were collected retrospectively, this is the first study that indicates that a sample of aversively treated former indentured child labors may transmit some of their hardships to the subsequent generation, plausibly with respect to their parenting styles.

4 General Discussion

The following section offers an overall discussion of this PhD project and its results. The findings are discussed with respect to their limitations and shortcomings. Thereafter, implications and possibilities for future research will be presented, followed by a closing and a general conclusion.

4.1 Overall Discussion

Resilience in elderly former indentured laborers?

The results from Paper 2 stand in contrast to previously reported findings from other samples or other investigated tissue samples (Kiecolt-Glaser et al., 2011; Ladwig et al., 2013; Savolainen et al., 2014; Shalev et al., 2012). This raises hope an alternative post-hoc assumption, indicating that the here investigated subsample of trauma survivors consists of a resilient subsample of survivors. More specifically, non-resilient individuals either died earlier or did not participate in the study, leading to a more resilient sample of former indentured child laborers that, despite childhood adverse events and PTSD, had longer buccal telomere length, and therefore, did not suffer from accelerated biological aging. In order to investigate this post-hoc assumption, that our participants might constitute a sample of particularly resilient individuals, participants were divided into a group with longer mean buccal telomere length and a group with shorter mean buccal telomere length by a median split. These analyses did not reveal any significant associations between buccal telomere length and any other variable that could indicate mental, social or physiological functioning, which partially weakened this assumption.

Since the design of this study did not allow to control for survivor effects, and given that so far, there is no data from indentured child laborers that suggests that their traumatic events also lead to earlier mortality, it is not fully possible to either support or deny this assumption.

Political incentives that might bias participant's responses

Despite the possibility of a resiliency explanation, the findings from Paper 2 indicated that the participating former indentured child laborers reported very high levels of childhood trauma and psychopathology. This might partially stem from an ongoing political issue in Switzerland at the given time when data for the study was collected. The question of how former indentured child laborers should be compensated for their endured injustice by the government was and still is a political and social debate in Switzerland. It is therefore possible that these factors influenced the participants' responsiveness in such terms that some participants consciously or unconsciously aggravated some responses in order to raise awareness for the given political issue. Such tendencies may be unintentional, but might still lead to biased results, as indicated by studies of US military veterans (McNally & Frueh, 2013).

Potential methodological issues

Analyses from Paper 2 potentially suffer from a batch effect (Soneson et al., 2014). Due to the fact that the control sample was only recruited after the initial indentured child laborers sample, we were not able to simultaneously process both batches. Even though the quantitative polymerase chain reaction-protocol is reliable and precautions were taken to ensure validity of the results (O'Callaghan et al., 2008), a potential batch bias is possible.

Batch effects can occur when biological samples of different groups are handled differently or at different time points. While the protocols for handling the samples, theoretically, is the exact same for any given batch, minor variations can cause systematic bias to the given batch. By either mixing samples from different groups or running all samples together, batch effects can be controlled methodologically. However, even within-group analyses did not indicate confirmation of our general hypothesis (in neither the former child laborer sample nor the control sample), indicating that a lack of a negative association between childhood trauma and buccal telomere length cannot be attributed to a batch effect only.

Support for indirect trauma transmission

The findings from Paper 3 indicate that childhood trauma experiences, but not necessarily psychopathology, are indeed higher for offspring from former indentured child laborers. This is in line with a recent study by Roth et al. (2014), which found that rather than direct association between maternal trauma or maternal PTSD and offspring psychopathology, maternally experienced violence (i.e. the violence the mother experienced during her childhood) is associated with offspring psychopathology. The authors, therefore, suggested familial contextual factors, i.e. maternal rearing behavior or parenting capacities that might act as a transmitting mechanism of maternal adverse experiences. Findings from Paper 3 point towards the same direction, since parental rearing behavior of former indentured child laborers has been described as more dysfunctional than rearing behavior from control.

Parental rearing behavior was previously identified as an important mediator in other studies (Field et al., 2013, 2011). These studies suggested that parental overprotection is most important for offspring psychopathology. Findings from Paper 3,

on the other hand, suggest that mainly maternal punishment and parental emotionality (but not parental overprotection) is a key aspect of parental rearing behavior when it comes to transgenerational transmission of traumatic stress. These different findings may be explained by the fact that analyses by Field and colleagues were within-group analyses of Cambodian Khmer Rouge survivor offspring, while we compared two different Swiss samples. It is, therefore, possible that within trauma survivors, overprotection is the most important aspect of parental rearing behavior, but when focusing on differences between groups, punishment and emotionality are more relevant. While one expects within group variance in a given population, one does not necessarily expect differences in this dimension in our two samples, since the two groups shared most of their cultural frame of reference. The dimension of control and overprotection might be context- and culture-specific, and might be shared over the cultural context of our investigated groups.

Positive dimensions of psychological well-being in trauma research

In line with other recent research (Fossion et al., 2014), Paper 3 found a weaker sense of coherence in offspring of former indenture child laborers. It is important to note here that Fossion et al. (2014) applied the original construct of the sense of coherence by Antonovsky (1987). In Paper 3, a revised form of the sense of coherence questionnaire was applied. According to Bachem and Maercker, the Sense of Coherence Revised questionnaire holds more distinctive variance, a better factor structure and more stability over Antonovsky's (1987) sense of coherence. Regardless of instrument, these findings add to the growing body of literature that offspring from trauma survivors not necessarily suffer because of elevated psychopathology, but due to weakened resiliency capacities.

Mechanisms of trauma transmission

Findings from other studies (Field et al., 2013, 2011) and from Paper 3 suggest that parenting style might be a potential candidate for transgenerational transmission of trauma. Nevertheless it remains an open field of research which other potential psychological mechanisms might play an important role in trauma transmission. Other than Field and colleagues (2013, 2011), who also specifically mentioned parenting style as a central mediator between parental adverse events and offspring psychology, many authors remain relatively vague about the intermediate pathways. Given that Paper 3 had neither the sample size nor the methodological design to adequately conduct mediation analyses, it remains unclear if parenting style actually is as critical as suggested in this study.

Integration of conflicting findings

Many recent research groups make a case for the intergenerational transmission of adverse events and trauma (Daud et al., 2005; Entringer et al., 2011; Field et al., 2013; Kellermann, 2001; Roth, Neuner, & Elbert, 2014; Yehuda et al., 2005), while others present evidence against it (Sagi-Schwartz et al., 2003, 2008). It is important to note that Sagi-Schwartz and colleagues (2003, 2008) analyzed a sample of Holocaust survivors, a very specific and unique population of trauma survivors. Interpersonal variables like the social acknowledgement of the trauma (Mueller, Orth, Wang, & Maercker, 2009; Wagner, Keller, Knaevelsrud, & Maercker, 2012) or sociological variables like socio-economic status or ethnicity (Perilla, Norris, & Lavizzo, 2002) of the trauma survivor may have a significant influence on the intergenerational transmission of trauma. Many other findings, including those from Paper 3, stem from populations

that are very different in terms of the trauma context. It therefore remains unclear if the findings by Sagi-Schwartz et al. (2003, 2008) are confined to cultural context or trauma context.

4.2 Implications for Further Research

The here presented empirical studies both applied cross-sectional designs. In the case of Paper 2, this lead to a curtail issue, since the design did not allow to control for survivor effects. Further studies should therefore try to utilize longitudinal designs in order to account for intra-individual trajectories.

Despite the here presented findings from Paper 2, the current research suggests that childhood adverse events and PTSD are both associated with shorter telomere length later in life (e.g. Ladwig et al., 2013; O'Donovan, Epel, et al., 2011; Shalev, 2012). Specific mechanisms for these associations are still an active field of research. It would therefore be important that future studies not only focus on sole associations, but rather investigate the underpinning mechanisms involving other biomarkers of psychological stress, such as inflammation (O'Donovan, Pantell, et al., 2011) or oxidative stress (Zglinicki, 2000). This goes hand in hand with the above-mentioned call for more longitudinal designs in the field. Given that human experimental studies that allow for causal interpretation are very hard to execute (and often also unethical), well-designed prospective studies would allow for further insight in telomere dynamics in relation to psychological stress and trauma.

Closely associated with the above-mentioned point, it is important to note that telomere research is somewhat limited/restricted in its applicability to everyday life and further consequences. Even though it is important to learn the potential

consequences of trauma for telomere dynamics in order to better understand biological ageing, there are few conclusions one can draw from it, other than that adverse life events are unhealthy. But in order to help affected individuals, future research should focus more on potential interventions (e.g. targeting health behaviors; Puterman et al., 2010; Puterman, Lin, Krauss, Blackburn, & Epel, 2014) that either prevent telomere shortening or reverse the effect altogether.

Given the small sample size in Paper 3, findings must be interpreted with caution. Given that only very few families participated in the transgenerational study, data from different siblings within the same family were utilized. Even though their data proved to be statistically independent from each other, this approach is inherently problematic. Due to exposure to a shared environment, siblings are inherently not independent from each other. Even though not suggested in this study (due to low sample size), future study should account for the nested structure of the data. This would also allow investigating further hypotheses, such as whether birth order within a given family affects trauma transmission differently.

Future research should expand the potential horizon of transgenerational transmission to future research. This study and others (e.g. Field et al., 2013, 2011; Roth, Neuner, & Elbert, 2014) focus on the first subsequent generation of trauma survivors, indicating that despite earlier findings (Sagi-Schwartz et al., 2003), transgenerational trauma transmission could take place. If the conclusions from Paper 3 and others (e.g. Roth et al., 2014) hold true, then psychological trauma transmission is working on a holistic family level that potentially also incorporates future generations (i.e. third generations).

Furthermore, new research in the field of transgenerational trauma should focus on the specific context of childhood adverse events and childhood trauma of parents, and which aspects of these adverse events specifically affect the offspring generation. Most transgenerational research (e.g. Daud et al., 2005; Fossion et al., 2014; Roth et al., 2014), including the research presented in Paper 3, focuses on man-made trauma, while no study was found that also analyzed aspects of natural disasters, accidents or medical emergencies. And even traumata that are man-made are very different from one another. For instance, it is not clear how the cultural and social acknowledgment of a trauma potentially affects the generational transmission of said trauma. It would therefore be vital that future studies try to account for the cultural context of a trauma population.

4.3 Final Conclusion

The above cited research as well as the here presented papers have shown that adverse and traumatic childhood experiences can shed both a biological (Ladwig et al., 2013; O'Donovan, Neylan, Metzler, & Cohen, 2012) as well as psychological (Burri et al., 2013) "long shadow" (Brent & Silverstein, 2013) on the affected individuals and their close ones (Entringer et al., 2013; Field et al., 2013; Roth et al., 2014; Yehuda et al., 2005). This thesis reflected on the issue of childhood adverse events from the two perspectives of biological consequences as well as transgenerational aspects.

With regard to the former of the two, the following final conclusions can be drawn: Even though telomere research is still an active field of research, the majority of researchers in this field agree that telomere erosion is likely to take place due to psychological traumatic stress (e.g. Jergović et al., 2014; Shalev et al., 2012; Tyrka et al.,

2010). However, it is the processes underlying these observations that we need to understand more thoroughly. If indeed biological aging – as indexed by telomere length – is accelerated through psychological stress, this means that, inevitably, other biological processes must underlie this acceleration. Subsequently, it should be these mechanisms that research should focus on in the long run. Finding out that biological aging is affected by psychological stress might be an important first step, but it can hardly offer specific explanation to help treat the affected individuals. Therefore, further research should aim at understanding the processes involved, thereby possibly facilitating ways of intervening or even averting them. Research on mediators of telomere erosion, such as inflammation (O'Donovan, Pantell, et al., 2011) or on developing health interventions in order to prevent telomere erosion in the first place (Puterman et al., 2010) constitute promising approaches in the right direction. The reason for this is that determining those mediating processes are of immediate use for the affected individual, as they can be targeted via lifestyle interventions and changes.

When looking at transgenerational transmission of trauma, however, the findings of the present thesis suggest that adverse childhood experiences do not only bear consequences for the first generation, but also potentially influence the well-being of the entire familial system. Presumably via parental rearing behavior and communictaion, trauma appears to transfer indirectly onto the next generation, thereby raising support for systemic therapies, which take the whole familial system into consideration as well as topics on the positive spectrum of psychological well-being, such as an individual's sense of coherence. One has to bear mind that psychological disorders hardly exist in an isolated form, but usually occur in the context of complex psychosocial and cultural environments, i.e. families and/or close relationships.

Additionally, future studies and clinical work should take into consideration that transgenerational trauma does not equal transgenerational trauma. That is, there is no such thing as "the trauma", and every individual is affected by its traumatic experiences in a different manner. Among others, one should take into account the context of the traumatic event, ranging from cultural context (Perilla et al., 2002) to social acknowledgment as a trauma survivor (Maercker & Müller, 2004) etc. Hence, the fact whether or not the traumatic event is openly addressed in a family (or even in the community) or rather avoided due to social stigmata inevitably also affects the way in which the second generation will deal with their "psychological heritage".

5 Paper One: Transgenerational Effects of PTSD or Traumatic Stress: Do Telomeres Reach Across the Generations?

(Küffer, Burri, & Maercker, 2014)

Abstract

Traumatic stress can alter allostasis and therefore mediate the development of psychological disorders. Recent evidence from molecular studies has shown that telomere length - a measure of cellular aging - is strongly influenced by a broad spectrum of stress. Telomere erosion might be accelerated by traumatic stress, and traumatic stress has shown to be associated with the risk of developing chronic diseases like cancer, cardiovascular diseases and immunologic conditions.

Aim: The biological pathways between psychological stress and psychological disorders or physiological diseases are widely unknown. Some experimental studies in animal models and longitudinal studies in humans have investigated the transgenerational consequences of psychological stress on telomere length biology. Telomere length inheritance might provide an additional molecular mechanism for the germ line transmission of environmentally induced phenotypic change and might offer a new biological framework for the multifactorial path etiology underlying stress-related disorders.

Procedure: Starting from the well-established allostatic load model, this article reviews theoretical and empirical work from animal models and humans in the field of telomere biology in association with traumatic stress, childhood trauma and post-traumatic stress disorders. Further it reviews recent approaches on telomere length

inheritance, and combines these findings with transgenerational research of post-traumatic stress disorder biology.

Conclusion: A better understanding of the transgenerational mechanisms underlying common diseases might ultimately help disease prevention of stress related disorders in subsequent generations.

Keywords: Telomere length; Allostasis; Transgenerational; Stress; Trauma; Post-traumatic stress disorder; PTSD

Introduction

In the last few decades, research has focused strongly on identifying biomarkers that are associated with stress- and trauma-related disorders. This research provided the empirical evidence for several etiological models that explain the pathways from physiological symptoms to psychological disorders and vice versa. One of the best-known is McEwen and Stellar's allostatic load (AL) model, which describes the multidimensional effects of physiological and psychological stress (Juster et al., 2010; McEwen & Stellar, 1993; Picard, Juster, & McEwen, 2014). In the AL model, an organism's need to meet its external demands (i.e., environmental influences perceived as stress) is termed allostasis.

This short review discusses the possibility of transgenerational inheritance of stress- and trauma-related telomere length (TL) shortening as a molecular outcome according to the AL model. Unlike previous reviews on TL and stress (Price, Kao, Burgers, Carpenter, & Tyrka, 2013; Shalev, 2012; Shalev et al., 2013), we aim to integrate the idea of transgenerational aspects in TL biology in relation to stress exposure from animal models and from studies with human participants. We will first provide an introduction to telomere biology (i.e. the function of telomeres, their erosion processes, and their tissue stability) and then summarize recent research efforts that may introduce TL as a marker for stress-related disorders. This article will have a special focus on studies investigating post-traumatic stress disorder (PTSD) and childhood adversities and/or trauma, representing both models for conditions specifically associated with elevated amount of lifetime stress. Finally we will present a number of findings from transgenerational telomere biology and associate it to stress-related research (including traumatic stress) to present a new hypothesis in the field of psycho-biological research: TL shortening is not only a primary result of psychological

stress, but also possibly a biological mediator for stress inheritance to subsequent generations. Overall, this short review addresses a new aspect of the biological framework and etiology of stress-related disorders and explores implications for the prevention.

Chronic Stress and Allostasis

Allostasis represents the sum of chronic exposure to heightened fluctuating or repeated environmental challenges and therefore offers a biological paradigm for the effects of stress on an organism's psychobiologic well-functioning. The model describes three levels of outcome (McEwen & Stellar, 1993). The primary effects refer to a molecular level, in which cellular activities (e.g., gene-expression, receptors, enzymes, etc.) are affected. The second outcome level defines sub-clinical levels of physiological parameters (e.g. insulin, glucose, cholesterol, systolic and diastolic blood pressure, etc.), eventually leading to a range of stress-related diseases. The last outcome level represents the final stage of physiological deregulations and diseases (Juster et al., 2010b). According to the model, even small deregulations to molecular levels may lead to disadvantageous consequences in the long term (Juster et al., 2010b). Within the AL model, molecular biomarkers are classified as primary effects, and play a fundamental role given that they appear early in the development of psychopathology. As such, the identification of relevant biomarkers of stress is particularly important to elucidate the interplay between nature and nurture of stress-related disorders.

To date, many molecular markers have already been investigated with the spectrum reaching from genetic variations like single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), to epigenetic variations like CpG-rich or -poor regions with distinctive DNA methylation patterns (Ressler et al., 2011). Telomere

length (TL) represents another such candidate for molecular variation that seems to be especially prone to environmental influences. A number of working groups have confirmed the association between psychological stress and TL-shortening (Epel et al., 2004; Kananen et al., 2010; Kiecolt-Glaser et al., 2011; Malan et al., 2011; Tyrka et al., 2010). In brief, the findings suggest that the more an individual is exposed to chronic stress, the shorter the individual's telomeres are.

Structure and Function of Telomeres

Telomeres were first described in the Journal of Molecular Biology by Elisabeth Blackburn and Joseph Gall in 1978 (Blackburn & Gall, 1978). They are the protective caps located at the ends of the 23 human chromosome pairs and at the ends of other eukaryotic chromosomes, made of repeats of specific nucleotide sequences organized in tandem arrays. In humans the telomeric sequence is 5'-TTAGGG-3'. The number of repeats varies greatly between species and between and within subjects (see below). Telomeres are involved in maintaining genomic stability and regulating cellular proliferation (O'Sullivan & Karlseder, 2010). They have been shown to inhibit chromosomes to form end-to-end fusions by preventing the cell from identifying telomeres as DNA-double strand breaks (Jain & Cooper, 2010; O'Sullivan & Karlseder, 2010).

TL has a strong genetic basis with heritability estimates ranging from 34% to 82% (Njajou et al., 2007; Slagboom, Droog, & Boomsma, 1994). A meta-analysis looking at six populations including over 19'700 subjects aged 17 to 99 years found a mean heritability rate of 70% (Broer et al., 2013). Findings from quantitative trait linkage studies further identified several putative loci for TL in several genes, such as the telomerase RNA component *(TERC)*, the Telomerase Reverse Transcriptase *(TERT)*, the

nuclear assembly factor 1 ribonucleoprotein *(NAF1)*, oligonucleotide/oligosaccharide-binding fold containing 1 *(OBFC1)* or the regulator of telomere elongation helicase 1 *(RTEL1)* (Codd et al., 2013; D. Levy et al., 2010; Simon et al., 2006; Vasa-Nicotera et al., 2005).

Telomere Shortening and Erosion

In somatic cells telomeres progressively shorten with each mitotic division because of the inability of DNA polymerase to fully replicate the 3'-end of the DNA strand. In other words, with each cell replication cycle a small fragment of DNA cannot be replicated due to the nature of the replication process itself. This inability of copying the last nucleic acids results in a loss of about 50 basepairs of telomere sequences per cell doubling. This process is known as the end-replication problem, which is partly held responsible for biological aging (Cech & Lingner, 2008; Harley, 1991; Levy et al., 1992). In order to prevent chromosomes from losing essential genetic information, telomeres close up the chromosomal DNA strands described above. Instead of shortening a gene, the chromosome only loses some of its TL. Thus, telomeres act as the mitotic clocks of our body, allowing a mitotic cell only to replicate itself for a certain number of times until its telomeres shorten down to a critical length and therefore lead to cellular senescence (Campisi, 1997). This was elegantly illustrated in a longitudinal study conducted on transgenic mice, where the rate of increase in the percentage of short telomeres was predictive of the mice' life expectancy (Vera et al., 2012), and where telomerase-deficient cells (see below) with single short telomeres caused an earlier onset of senescence (Abdallah et al., 2010).

With a specific enzyme our body can compensate for some of this TL erosion. Telomerase is a ribonuclearprotein which counteracts TL shortening by a specialized

reverse transcriptase adding the 5'-TTAGGG-3'oligonucleotides to the 3'-end of the DNA-strand (Greider & Blackburn, 1989; Yu, Bradley, Attardi, & Blackburn, 1990). Telomerase is not active in all mitotic cells and is particularly important in cells that depend on replication of vast numbers (e.g. sperm cells, stem cells or activated lymphocytes). It is also believed to play an important role in immortalizing tumor cells by allowing them to intently proliferate without substantial TL loss. Except in sperm cells and tumors – where telomerase can elongate telomeres – telomerase can only slow down TL erosion without preventing it completely (Gomez et al., 2012; Smogorzewska & de Lange, 2004).

TL erosion due to proliferation of mitotic cells is one important, though most probably not the only mechanism causing shortened TL. DNA-strand damages are another reason held responsible for TL erosion (von Zglinicki, 2002). Apart from other exogenous sources that may damage molecular structures (e.g. UV-light, radiation, ozone etc.) telomeres have also been found to be affected by oxidative stress. It occurs when the enzymatic and non-enzymatic antioxidants fail to fully neutralize reactive oxygen species (ROS) (Monaghan et al., 2009). ROS are chemically instable molecules (e.g. superoxide, hydrogen peroxide, hydroxyl radical, nitric oxide), which are highly reactive to their cellular environment and tend to damage DNA by binding to nearby molecules (Corral-Debrinski et al., 1992; Fiskum et al., 1999). Monaghan et al. (2009) describe the effects of oxidative stress as a homeostasis between ROS and antioxidants. Oxidative stress occurs when the balance between the two agents shifts in favor of ROS. Hence, while ROS are responsible for the damage on the DNA, information about both agents are necessary in order to comprehend the picture (Monaghan et al. (2009) provide further details on the interaction between ROS and antixidants). Oxidative stress is particularly important as a damaging mechanism because psychological stress

has been shown to reduce antioxidative activity and to increase indices of ROS (Gidron, Russ, Tissarchondou, & Warner, 2006).

Beside these mechanisms – cellular turnover and DNA-strand damages due to ROS – other key players might contribute to TL shortening. Inflammation has been associated with TL loss, though pathways are not yet fully understood. One plausible hypothesis is that inflammation increases by moderating the metabolic demands. Hence, inflammation may increase cellular turnover and metabolism, leading to more ROS due to more mitochondrial activity for example. Studies have repeatedly shown an association between inflammation markers and progressed TL erosion, indicating the involvement of inflammation in TL erosion (Goronzy, Fujii, & Weyand, 2006). Another plausible hypothesis is that the immune activation itself leads to shorter TL by the rapid recruitment and differentiation of younger immune cells. Figure 3 summarizes the described pathway between psychological stress and TL erosion.

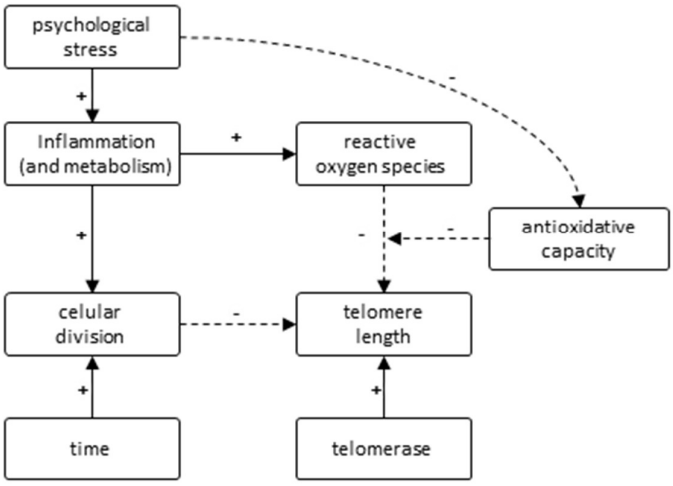

Figure 3. *Pathway-model of TL erosion*

Note. Pathways between stress and telomere erosion are currently still an active field of research. Plausible influence leads over oxidative stress and inflammation. Inflammation my increase cellular turnover and produce more reactive oxygen species, because of more metabolism. Antioxidative capacities neutralize ROS, while psychological stress decreases antioxidative capacities. Arrows indicate correlations, while minus signs on dashed arrows indicate negative correlation and plus signs on solid arrows indicate positive correlations.

It is important to note that it is not inflammation per se seems to shorten TL, but more likely the cellular renewal and increased metabolism, which most likely is induced by inflammation, is held responsible for the shortening. Further note that these two hypotheses are neither mutually exclusive, nor the only plausible mechanisms for TL erosion. They rather represent two complementary mechanisms to explain the implication of inflammation in TL erosion.

Telomeres in Different Tissues

Stability between TL in different healthy tissue samples and over time is not yet clearly determined and remains an area of active research. So far, only few studies have

compared TL concordance across different tissues (Friedrich et al., 2000; Kimura et al., 2010; Nakamura et al., 2002; Okuda, 2002; Thomas, O' Callaghan, & Fenech, 2008; Youngren et al., 1998), and to date no study observed TL dynamics in different tissue samples across multiple time points to compare TL dynamics between tissues over time. Still, some cross-sectional studies report synchrony over different tissues. One study by Kimura et al. (2010), for example, reported significant correlations between TL of hematopoietic progenitor cells from newborn umbilical cord blood samples and TL from lymphocytes or granulocytes, with r´s ranging from r =.88 to r =.94. In a second phase of their study, they showed that correlation between granulocytes TL and leukocyte TL were high at birth (r =.98) and stayed strong different age groups (r = .98). These results derived from cross-sectional data of a cohort with 24 subjects (age 22 to 34 years) and another cohort with 400 subjects (age 0 to 100 years) (Kimura et al., 2010). Some studies implied TL synchrony between different tissue samples across different stages of an organism´s development. A comparison of tissue samples from 13 different organs in 11 aborted fetuses (gestations weeks 15-19), for example, found no significant differences of mean TL across the tissues, with mean ranges being less than 1 kilobase for all samples (Youngren et al., 1998). Similarly, another study conducted on newborns found a strong correlation (r =.89) between TL of white blood cells and TL of umbilical artery cells (Okuda, 2002). And in two post-mortem samples of 41 and 21 subjects from 0 to 101 years of age, Nakamura reported significant associations in lingual epithelium cell TL and epidermis TL (r =.84 and r =.93, respectively) (Nakamura et al., 2002). By additionally controlling for donor age, Friedrich and colleagues found significant correlations in tissue samples from 9 patients (age 75-95 years) between epidermis TL and leukocyte TL (R^2 =.79), epidermis TL and synovial sample TL (R^2 =.71), and synovial sample TL and leukocyte TL (R^2 =.54) (Friedrich et al., 2000). Whilst

these studies point towards TL tissue synchrony, one study failed to find evidence for such synchrony. Thomas, O'Callaghan, and Fenech (2008) found no significant correlation between buccal cell TL and whole blood cell TL in three different samples, including 30 young adults (age 18-26 years), 26 healthy old adults (age 64-74 years) and 54 clinically diagnosed Alzheimer's patients (age 58-93 years) respectively. While there are several indications that there is a substantial correlation between TL in different tissues, it is important to note that most studies on the subject of tissue-correlation incorporated only small sample sizes. Combined with the small number of studies in this particular field, satisfying conclusions cannot yet be drawn on how strong synchrony is over different tissue samples.

Stress Related Disorders over Subsequent Generations and TL

The Effects of Stress, Traumatic Stress and PTSD on TL

Telomeres are not only considered an index of cellular age but have also been associated with physical morbidity and mortality. Associations between shorter TL and a number of disorders have been reported, such as cancer [(Willeit et al., 2010; Willeit, Willeit, Kloss-Brandstätter, Kronenberg, & Kiechl, 2011), immune (O'Donovan, Pantell, et al., 2011), and cardiovascular diseases (Epel et al., 2009; Fitzpatrick et al., 2011). Besides these physical disorders, psychological conditions (i.e. psychological stress) have become more important in research over time.

A recent experiment using an animal model demonstrated the impact of early post-natal life stress and subsequent TL erosion in erythrocytes (Herborn et al., 2014). The authors divided a total of 114 European shag chicks in three groups: an unhandled control group of 36 chicks, a handled-CORT group of 42 chicks and a handled-oil group

of 36 chicks. The CORT-group received oral administered corticosterone to elevate glucocorticoid hormone levels, while the handled-oil group received fish oil to induce a comparable amount of exogenous stress levels. The chick's erythrocytes TL were measured 10 days after hatching and 20 days later after subsequent stress manipulation. Though there was no significant difference between the two stress groups, both groups showed significant shorter telomeres compared to the control group.

Recent studies in humans, although all non-experimental, suggest that stress has a similar impact on human TL biology. Epel and colleagues (2004) demonstrated a significant association between lifetime stress and shortened telomeres. Within the last decade, researchers have highlighted further facets of stress. A considerable number of studies found significant relationships between stress, traumatic stress (and its consequences, such as PTSD) and TL, including experimental confirmation in animal models (Herborn et al., 2014; Kotrschal et al., 2007), and a series of studies demonstrating this in children as well as adults exposed to early adversity. The following paragraph provides a summary of these findings.

In a prospective longitudinal study with two measurement points conducted on 236 children at the age of 5 and 10 years, Shalev and colleagues (2012) showed that children with more exposure to violence also had significantly more TL erosion (i.e. shorter TL at age of 10 compared to the age of 5 years) compared to children with less exposure to violence. Using a prospective design including repeated measurements, this study provided evidence that childhood stress might have impaired telomere maintenance in the long run. According to the authors, TL erosion might have been a direct consequence of chronic childhood stress rather than the product of moderating/mediating variables (such as poor physical health in adulthood due to

childhood maltreatment). In other words, telomere erosion does not seem to be the cause of later health problems, but a proximal effect of maltreatment itself. Also in 2012, Drury published their study on the association between foster care placement and TL. The authors considered children living in institutions as a model for early adversities. They investigated a total of 136 children allocated to one of six institutions in Bucharest (Romania). For TL baseline assessment, buccal swaps were collected between 6 and 30 months of age, and compared with TL from 54 months of age. The percentage of time spent in institutional care was used to predict TL. Percent time spent in the institution was significantly and negatively associated with TL and remained so, even after controlling for potential cofounders like gender, ethnicity, low birth weight and age at telomere collection. Recently, Drury and colleagues (2014) also published a cross-sectional study about 80 (age 5-15 years) children who were exposed to family violence and disruptions. After controlling for age, sex, maternal education (as a proxy for socio economic status), and paternal age of conception (PAC), they found that the number of adverse life events were significantly associated with TL. Children with no exposure to adverse family events had significantly longer TL than those exposed to one, two or more events. Savolainen et al. (2012) aimed to replicate the findings of Drury et al. (2012) in a sample of 1486 old adults (mean of 61.5 years age at the time of tissue collection), where 215 had been separated from their parents in childhood. Additionally to the parental separation, they assessed self-reported physically and emotionally traumatic experiences throughout the lifespan with the Traumatic Experiences Checklist (TEC). Even though they did not find a significant association between LTL and the early life separation, emotional or physical trauma over the total sample, the group found shorter TL in the participants who were separated from their parents in childhood and reported traumatic experiences in their lifespan. In line with this model,

a study conducted by O'Donovan et al. (2011) on 43 patients with chronic PTSD and 47 control subjects found significant associations between PTSD diagnosis and TL shortening. In the same study, a significant association between childhood trauma (CT) and telomere shortening was found within the PTSD group. Additionally, exposure to cumulative CT was linearly associated with shorter TL. CT was assessed dichotomously by the participants' perception of life threat a er experiencing physical neglect, family violence, physical abuse, forced sexual touch, or forced sexual intercourse before the age of 14 years. In a relatively large sample of 4441 women (aged 41-80 years) from the United Kingdom mean TL was significantly associated with adverse experiences during childhood, also a er controlling for various covariates (e.g. social class, obesity, smoking status, preexisting disease, physical health, and self-reported health) (Surtees et al., 2011). In this study the number of adverse events until an age of 17 years reported by the participants predicted whole blood TL negatively. Similar findings were reported by Kananen et al. (2010), who investigated 321 individuals with anxiety disorder and matched controls (age 30-87 years). They found that the total cumulative number of childhood adversities was significantly associated with TL by the means of a linear relationship: the more adversities subjects reported, the shorter their telomeres were. Providing further support to these childhood adversities-TL erosion findings, childhood maltreatment was significantly associated with TL in a sample of 31 adults (Tyrka et al., 2010). Tyrka and colleagues (2010) administered the childhood trauma questionnaire (CTQ) to subjects with no current mental disorder to check associations between CTQ and TL. According to their CTQ scores, participants were allocated to a no-maltreatment group when they reported no maltreatment or only moderate scores in the five CTQ subscales "physical abuse", "sexual abuse", "emotional abuse", "physical neglect" and "emotional neglect". Participants with moderate to severe scores were selected for the

maltreatment group accordingly. Like the other authors, Tyrka et al. (2010) found shorter TL within the participants with childhood maltreatment (e.g. the maltreatment group).

It is not only adverse childhood experiences that seem to be significantly associated with TL. In a seminal study conducted on a sample of 3000 subjects, PTSD was significantly associated with shorter TL (Ladwig et al., 2013). PTSD was assessed with the Posttraumatic Diagnostic Scale and the Impact of Event Scale, and participants were assigned to three different groups. Depending on their PTSD status, participants were assigned to three different groups (i.e., no PTSD, partial PTSD, and full PTSD). Even though significant differences could only be detected between the no PTSD group and the two other groups (but not between the partial PTSD, and full PTSD group), the findings point towards a linear association in terms of a dose-response model: the stronger the PTSD symptomatology, the shorter the mean TL. Finally, a longitudinal study investigating the sequelae of rape in 64 females with PTSD, where of 23 were also diagnosed with major depressive disorder (MDD), the authors provided further support to the association between TL and PTSD (albeit not between TL and MDD) (Malan et al., 2011). In an investigation conducted on 650 volunteering US army special operation units (with exposure to combat experiences), Zhang et al. (2014) found lower LTL in participants who scored positive for PTSD, compared with those who scored negative for PTSD. In 2014, Shalev et al. (2014) conducted an analysis in a subsample of 827 participants from the Dunedin Study (N = 1037 original birth cohort), where leukocyte TL and psychometric data was available. Their results showed that the persistence of internalizing disorders (i.e. depression, generalized anxiety disorder and PTSD) among men predicted shorter leukocyte TL at age 38 in a dose-response manner. Where leukocyte TL data was available at age 26 and later at age 38, they were able to predict

TL erosion over the given time. Male participants who developed one of the previous described disorders over the course of the 12 years, showed significantly stronger decline in TL than there healthy controls. In order to explain the given sex difference in their study, the authors cited consistent findings which found that men with mental disorders, are at higher risk for mortality than females with mental disorders and that physiological and biochemical systems, which are associated with higher oxidative stress and inflammatory markers, might be more affected in men with mental disorders. Further they hypothesized that the sex difference might be due to the fact, that the study period covered the women's reproductive phase, which might act as a protective factor for TL erosion. Estrogen is thought to have mitochondrial anti oxidative properties. Further, telomerase expression and activity is increased in the presence of estrogens. Therefore, the authors suggest, women might be less susceptible to disorder-linked telomere erosion during their reproductive phase.

Overall, these study results suggest that shortened TL might be the biological consequences of stress exposure during lifetime. Together with other risk factors (see below) TL might represent a plausible biomarker for PTSD and disorders specifically associated with stress. Nevertheless, it remains unclear how adverse life events affect TL biology and further studies are needed to elucidate the mechanisms of this trauma-TL association.

Intergenerational Transference of Stress-induced Psychopathologies

It is well established that environmental exposures such as stress, trauma or exposure to toxins during fetal and early postnatal development can lead to an increased incidence of adult-onset diseases (Bale et al., 2010; Barker, 2007; Godfrey et al., 2010; Jirtle & Skinner, 2007). However, the mediating biological mechanisms from

environmental stressors to individual disorders remain unclear. A recent study on mice demonstrated a potential mechanism, which might contribute to gene-environment interactions (Gapp et al., 2014). The authors suggested that small non-coding (snc) RNAs might play a causal role in transgenerational inheritance of traumatic experience by injecting purified sperm sncRNAs from traumatized mice (by a model of unpredictable maternal separation and unpredictable maternal stress; MSUS) into wild-type fertilized mouse oocytes. RNA injected mice showed significantly altered response to aversive conditions and even depressive-like symptoms. At the same time the authors investigated the glucose metabolism in the MSUS (F1) mice mentioned above, non-traumatized control mice (F1 controls), and the offspring of both groups (F2). Through glucose and insulin tolerance tests the authors suggested that the F2 MSUS mice showed insulin hypersensitivity and over all a hyper metabolism. These findings are in line with the in Figure 3 suggested pathways of TL erosion, since metabolism, indirectly induced through psychological stress, might lead to shorter TL. To our knowledge, there are no studies, which investigated those mechanisms in humans so far. But a limited number of studies have focused on transgenerational consequences of perceived parental stress and telomere biology.

In 2011, Entringer et al. investigated the association between leukocyte TL in a sample of 94 healthy subjects and their exposure to intrauterine stress (i.e., prenatal stress). Their parental stress exposure was a significant predictor for TL length, even after controlling for important potential confounders such as birth weight, early-life stress and current stress. In 2013, the same group conducted a study on 27 mother-newborn dyads in order to assess how early maternal stress levels during pregnancy (i.e. also prenatal) affect newborn leukocyte TL (Entringer et al., 2013). In this second study, the authors found further evidence for significantly shorter leukocyte TL in

newborns from mothers with greater stress exposure, indicating some sort of stress-related programming red in newborn TL. The authors discussed that stress-induced release of maternal hormones may lead to inflammation and oxidative stress mediation. As suggested by Gapp and colleges (2014), the male germ line might provide particular insights in the mechanisms of biological trauma- transmission. Allsopp et al. (1992) for example, reported that sperm telomeres in human - maintained by telomerase - increase 71 base pairs in length per year. That implies that paternal TL deserves further emphasis. While female germ cells develop and proliferate during the second trimester of gestation and afterwards rest in post mitotic stage (Stoop et al., 2005), spermatogenesis in testis starts after puberty and continues until death, and therefore- unlike oocytes – sperm cells rest mitotic and proliferating. Sperm cells would enter senescence very early if it were not for telomerase. Telomerase counterbalances the TL loss over time in healthy mitotic cells, such as sperm cells, leading to a constant sperm TL over time or even a slightly elongated sperm TL.

Previous studies have suggested that sperm TL of older men are longer than those of younger men (Allsopp et al., 1992; Kimura, Cherkas, et al., 2008). Given the correlation between sperm TL and paternal age, it seems evident that PAC or paternal age of birth (PAB) could serve as a proxy for the assessment of TL inheritance in subsequent generations. Several research groups have already used this procedure. Table 1 provides a brief overview of studies conducted on samples in which PAC/PAB was associated with offspring TL.

Broer (2013) and colleagues investigated not only TL heritability but also TL inheritance by investigating the correlation between offspring TL and parental TL. They checked for paternal and maternal effects separately and found a strong association with both parents (mother-offspring correlation of r =.42; father-offspring-correlation

of *r* =.33). Interestingly, the meta-analysis also indicated a positive association between PAB and offspring TL across five different samples (Table 1). The older the fathers were at the birth of their children, the longer the children's mean TL was. Several other research groups reported similar findings. In a study conducted on a sample of 2433 subjects (1176 men and 1257 woman) with an age-range of 35 to 55 years, PAB was positively associated with offspring TL [71]. In a study from 2005, Unryn, Cook, and Riabowol (2005) investigated 125 randomly chosen subjects (51 men and 74 women), and also found a strong association between PAC and offspring TL (*r* =.46). Njajou and colleagues (2007) reported heritability estimates of 44%. Consistent with the findings of the other research groups, they also observed a significant positive association between paternal TL and offspring TL and a significant PAC-offspring TL association in a subsample, and therefore for TL inheritance. Kimura, Hjelmborg, et al. (2008) observed a significant association between PAB and offspring TL in four different cohorts (resulting in a total of 3365 subjects. This was supported by findings from Arbeev, Hunt, Kimura, Aviv, and Yashin (2011), and Eisenberg and Kuzawa (2013).

Table 1. *Overview of different studies that have investigated the effect of parental age of conception or birth on TL*

References	Samples	N	Age *m(sd)/range*	PAC/PAB	Effect (*r* / *β*)	*p*
Broer et al. (2013)	Erasmus Rucphen Family	560	26.7 (4.1)	PAB	*β*(TL/y)= 0.0086±0.0038	0.024
	GRAPHIC Study	983	28.3 (4.2)	PAB	*β*(TL/y)= 0.0053±0.003	0.077
	Leiden Longevity Study	1587	35.5 (5.9)	PAB	*β*(TL/y)= 0.0029±0.0021	0.167
	The Netherlands Twin Register	1305	30.0 (4.6)	PAB	*β*(TL/y)= 0.0080±0.0031	0.01
	Queensland Institute of Medical Research	869	32.2 (5.3)	PAB	*β*(TL/y)= 0.0066±0.0048	0.0013
Eisenberg, Hayes and Kuzawa (2012)	Cebu sample (offspring)	1681	36 - 69-y	PAB	*β*(TL/y)= 0.0027±0.0013	> 0.0001
	Cebu sample (grand children)	234	21 – 23 y	GPAB	*β*(TL/y)= 0.0029±0.0027	0.038
Arbeev, Hunt, Kimura, Aviv and Yashin (2011)	Family Heart Study	2177 (995 m / 1182 w)	31-86 y	PAB	m: *β*(bp/y) =12.9±9.6; w: *β*(bp/y) =19.4±10.1	0.012 0.002
Kimura et al. (2008)	Framingham Heart Study	432 (235 m / 197 w)	18-80 y	PAB	m: *r*= 0.21 w: -	= 0.001 ns
	Family Heart Study	847 (355 m / 492 w)	32 - 84 y	PAB	m: *r* = 0.19 w: *r* = 0.14	0.0003 0.016
	Longitudinal Study of Aging Danish Twins	132 (44 m / 88 w)	73-94 y	PAB	m: *r* = 0.63 w: -	0.001 ns
	UK adult twin registry	1954 w	18-79 y	PAB	w: *r* = 0.17	0.001
De Meyer et al. (2007)	Asklepios study population	2433 (1176 m / 1257 w)	35–55 y	PAB	m: *β*(bp/y) =15.5±5.9 w: *β*(bp/y) =19.1±6.1	> 0.0001 > 0.0001
Njajou et al. (2007)	Amish families	229 (89 m / 140 w)	18-92 y	PAC	*β*(bp/y) = 10.4±8.44	0.05
Unryn et al. (2007)	Random subjects	125 (51 m / 74 w)	30 -80 y	PAC	*r* = 0.46	0.01

Note. PAC = parental age of conception; PAB = parental age of birth; GPAB = grandparental age of birth; TL/y = telomere length per years; bp/y = base pairs per years; m = men; w = women

TL and its Association with Transgenerational PTSD Susceptibility

Most research on transgenerational aspects of stress has focused on individuals suffering from PTSD. Familial accumulation of PTSD has led researchers to the conclusion that PTSD has a genetic component (Afifi et al., 2010; Stein et al., 2002; True et al., 1993). In other words, parental PTSD appears to be a salient risk factor for the susceptibility to develop a PTSD in subsequent generations. In a seminal study conducted on Holocaust survivors (N = 100) and a demographically comparable control group (N = 44), adult offspring of Holocaust survivors showed a significantly higher PTSD prevalence compared to the offspring of the control subjects (Yehuda et al., 1998). Analyses on a subset of the sample (N = 39 offspring of Holocaust survivors and N = 15 offspring of the control group) further showed a significant negative correlation between severity of parental PTSD and offspring urinary cortisol, as well as a strong correlation between offspring PTSD symptom severity and their cortisol levels (Yehuda et al., 2002), suggesting a biological, transgenerational link between parental and filial PTSD symptomology (Yehuda, 2002; Yehuda et al., 2002). In yet another follow-up study using a different sample, the same research group investigated the relationship between maternal cortisol levels (from 38 mothers directly exposed to the World Trade Center collapse) and cortisol levels from their one-year old infants. Lower cortisol levels were observed in both babies and their mothers who developed PTSD in response to September 11th compared with mothers who did not develop PTSD in response to the same event and their babies. Those findings might contain implications for the human telomere biology.

A meta-analysis by Costantini, Marasco, and Møller (2011) analyzed the effect of glucocorticoids on oxidative stress and noted that with an overall Perason's r =.55 the

implications of glucocorticoids for oxidative stress were significant. Haussmann, Longenecker, Marchetto, Juliano, and Bowden (2012) injected corticosterone into yolk of domestic chickens to test their hypothesis weather the exogenous corticosterone influences cellular ageing processes (i.e. oxidative stress and TL) during embryonic development. For outcome variables they analyzed the relationship between reactive oxygen metabolites and total antioxidant capacity in two treatment groups (high and moderate corticosterone injection) and one control group. After injection and incubation, seven chickens per group remained for analyzes. Birds with elevated prenatal corticosterone showed more reactive oxygen metabolites after hatching and less total anti oxidative capacity compared to controls. This differences between the groups disappeared 40 minutes post-hatch, but all groups showed significantly more reactive oxygen metabolites and less total anti oxidative capacity. At the same time they found significantly shorter TL in blood plasma of birds with higher corticosterone injections. This study was most likely the first work that significantly demonstrated the prenatal effects of glucocorticoids on TL dynamics. But together with the findings on the influence of maternal stress on offspring TL (Entringer et al., 2011, 2013; Gapp et al., 2014), the new findings from animal models (Haussmann et al., 2012) might suggest a link between the 4 factors cellular metabolism, inheritance, telomere biology and transgenerational consequences of PTSD and other stress related disorders.

Evidence, however, is not completely consistent and some studies question trans generational consequences of PTSD (Sagi-Schwartz et al., 2003, 2008). Sagi-Schwartz et al. (2003) investigated a sample of 196 female subjects (48 Holocaust survivors and 50 controls, each had one daughter) to address trans generational effects form mothers to their daughters. They found significant impacts of Holocaust in the first generation but not the second generation. In a meta-analytic approach from 2008 integrating 13 non-clinical

samples with 1012 subjects the authors focused on a transmission of trauma to the third generation (Sagi-Schwartz et al., 2008). Like in their study on secondary traumatization, the group stated no effect of tertiary traumatization of Holocaust. Nevertheless, in both studies the authors focused on psychological outcomes only (e.g. attachment-style, cognitive worries, anxiety, general adjustment, aggression) but not on biological indicators like Yehuda et al. (2002) in their study on cortisol levels of adult offspring. It is therefore of great importance to investigate whether TL is affected by trans generational effects of PTSD.

Discussion

TL shows high inter- and intra-individual variability, and tends to be crucially involved in the development of many physiological and psychological conditions, specifically when associated with stress. Besides the importance stress-related research, TL might be a useful biomarker in transgenerational psychobiological research and a potential target for a number of medical and bio psychosocial interventions.

Three findings underline the idea that TL is an optimal candidate for transgenerational PTSD research: a) TL is highly inherent and heritable, stressing a transgenerational approach b) high amounts of stress preceded the development of PTSD, which is also likely to influence TL maintenance as discussed above, and c) PTSD induces subsequent life-stress and arousal, which is why it is also likely to contribute to TL maintenance.

e biological framework for stress-TL associations has yet to be investigated, since there are currently two hypotheses, which might explain the association between TL erosion and psychological variables. The first hypothesis refers to an organism's elevated arousal during

the experience of stress due to its inflammation (Goronzy et al., 2006). More arousal leads to more bodily activity and metabolism that leads to increased cellular turnover. The elevated cellular turnover leads to more DNA replication and therefore faster TL erosion over time. The second hypothesis states that ROS (as molecular byproducts of metabolism) are responsible for DNA damage (von Zglinicki, 2002). Besides other factors, psychological stress reduces anti oxidative activity. These two models are not mutually exclusive, and it is likely that both have a substantial part in the processes of TL erosion. More research is needed to complete the biological framework behind psychological stress and TL erosion.

It is likely that besides "normal" TL inheritance, TL erosion due to psychological stress might also be passed on to subsequent generations and thus leads to physiological and mental disadvantages in the filial generations. In other words, illness and diseases in subsequent generations could be due to direct multigenerational exposure to environmental factors, particularly to stress and trauma. However, it remains unclear whether transgenerational TL elongation due to higher PAC could compensate for such TL erosion. Clearly, further research is needed to understand the biological transgenerational effects of stress and trauma to prevent future adverse health outcomes in subsequent generations. So far, few studies have investigated the role of maternal prenatal stress-exposure in TL maintenance in their newborns. The studies consistently support the hypothesis that increased physiological activation (e.g. due to stress) or arousal can affect in utero telomere biology. However, it remains unclear whether antenatal stress (i.e., parental stress-exposure before conception) moderates germ-line transmission of TL. Though the significance and importance of PAC has been investigated in healthy subjects, it remains to be clarified whether the PAC-effect is fully or partially compensated in families

with strong TL erosion due to high antenatal stress-exposure and whether maternal antenatal stress is transmitted to subsequent generations by the means of TL maintenance.

In conclusion, by deepening our understanding of the biological mechanisms underlying TL maintenance, and the relative importance of stress exposure for the trans generational development of stress- related conditions and diseases via TL, telomere research may lead to the improvement of not only more appropriate disease treatments but also more suitable prevention approaches.

6 Paper Two: Post-Traumatic Stress Disorder, Childhood Adverse Events and Buccal Cell Telomere Length in Elderly Swiss Former Indentured Child Labors

(Küffer. O'Donovan, Burri, Maercker, 2016)

Abstract

Posttraumatic stress disorder (PTSD) is associated with increased risk for age-related diseases and early mortality. Accelerated biological aging could contribute to this elevated risk. The aim of the present study was to assess buccal cell telomere length (BTL) – a proposed marker of biological age – in men and women with and without PTSD. The role of childhood trauma was assessed as a potential additional risk factor for shorter TL.

The sample included 62 former indentured Swiss child laborers (age: M = 76.19, SD = 6.18) and 58 healthy controls (age: M = 71.85, SD = 5.97). Structured clinical interviews were conducted to screen for PTSD and other psychiatric disorders. The Childhood Trauma Questionnaire (CTQ) was used to assess childhood trauma exposure. Quantitative polymerase chain reaction was used to measure BTL. Covariates include age, sex, years of education, self-evaluated financial situation, depression, mental-, and physical functioning.

Forty-eight (77.42%) of the former indentured child laborers screened positive for childhood trauma and 21 (33.87%) had partial or full-blown PTSD. Results did not support our hypotheses that PTSD and childhood trauma would be associated with shorter BTL. In

fact, results revealed a trend towards longer BTL in participants with partial or full PTSD (F[2,109] = 3.27, p = .04, η^2 = 0.06), and longer BTL was marginally associated with higher CTQ scores (age adjusted: β = 0.17 [95% CI: -0.01 – 0.35], t = 1.90, p = .06). Furthermore, within-group analyses indicated no significant association between BTL and CTQ scores.

To our knowledge this is the first study exploring the association between childhood trauma and BTL in older individuals with and without PTSD. Contrary to predictions, there were no significant differences in BTL between participants with and without PTSD in our adjusted analyses and childhood adversity was not associated with BTL. Possible explanations and future research possibilities are discussed.

Keywords: PTSD, Childhood adverse experiences, old adults, buccal telomere length, quantitative polymerase chain reaction, Swiss indentured child laborers

Background

Until the late 1970s, it was a common practice in Switzerland to have orphans, children of single-parent families, or even children from divorced or separated parents removed by the authorities from their family environment into foster care. Unlike typical foster care, the children were specifically sent into farmers' homes or other types of working class families in rural areas to work. Nowadays, this would essentially be described as indentured servitude and would clearly be classified as child labor. Historic studies have documented the harsh environment in which these individuals grew up, reporting that a large proportion of the children were regularly beaten, emotionally and sexually abused, and that some of them died or were murdered (Leuenberger & Seglias, 2008). Most of these Swiss indentured child laborers [In German: "Verdingkinder"] are now in late life and studies have reported high prevalence of adverse childhood experiences and poor mental health (Burri et al., 2013; Kuhlman et al., 2013).

Telomere length (TL) has been proposed as one of the mechanisms of early life stress effects on physical health (Kendall-Tackett, 2009). Telomeres are involved in genome stability and the regulation of cellular proliferation. TL varies between and within species, and within subjects over time, as well as between different cell populations (Blackburn 1991). Telomeres shorten with each mitotic division, because of the inability to fully replicate the 3'-end of the DNA strand (Hayflick, 1965). Telomeres have also been shown to shorten exogenous sources (e.g., UV-light, radiation, ozone etc.), oxidative stress, and/or inflammation (O'Donovan, Pantell, et al., 2011; Thomas von Zglinicki, 2002). A considerable number of studies – including experimental animal models – have found significant relationships between stress, traumatic stress (and its consequences), such as

posttraumatic stress disorder (PTSD) and TL (Carroll et al., 2013; Herborn et al., 2014; Kotrschal et al., 2007; L Zhang et al., 2013).

Further studies have now linked adverse childhood events (ACEs) with TL. A study on 43 patients with PTSD (including 18 PTSD patients with multiple categories with of childhood trauma) and 47 control subjects found significantly shorter leukocyte TL (LTL) in participants with PTSD compared to control subjects, only if the participants with PTSD also suffered from exposure to multiple categories of childhood (O'Donovan, Epel, et al., 2011). In 2014, for example, the prospective longitudinal Dunedin Study conducted on *n* = 1037 subjects 11 years of age showed that persistent internalizing disorders in men - but not in women -, predicted LTL at 27 years later in a dose response manner (i.e. the more years they reported to have suffered, the shorter were their LTL: Shalev et al., 2014). In another cross-sectional study by Drury and colleagues including 80 children (age 5-15 years) exposed to family violence, the authors found that the number of adverse life events was associated with shorter buccal cell TL (BTL; Drury et al., 2014). Savolainen studied a sample of 1,486 adults (mean age of 61.5 years) at the time of tissue collection (Savolainen et al., 2014). They assessed separation from parents in childhood (*n* = 215), as well as self-reported physically and emotionally traumatic experiences throughout the lifespan. Even though they did not find a significant association between LTL and either early life parental separation or emotional and physical trauma across the full sample, they found shorter LTL in the participants who were separated from their parents during childhood and who also reported traumatic experiences. A study of a sample of 3,000 subjects, PTSD alone was associated with shorter LTL (Ladwig et al., 2013). One study that investigated LTL in 496 elderly individuals (mean age of 70.6 years; *SD* = 7.4 years) found no significant association with childhood abuse, recent negative life events or loneliness (Schaakxs et al., 2015). Only

early separation from parents was marginally negatively associated with shorter LTL. Hence, it remains unclear whether individuals with repeated and extensive exposure to ACEs potentially suffer from accelerated biological ageing, as indexed by TL, or not, and to the best of our knowledge, no studies have examined the effects of ACEs on BTL in later life in individuals with and without PTSD.

As evidence for the potential causal role of ACEs in shortening telomeres, some studies have reported a faster rate of decline in TL in children experiencing adversity. In one prospective longitudinal study of 236 children, Shalev and colleagues found that children with more exposure to violence showed significantly more BTL erosion from age 5 to 10 years compared to children with less violence exposure (Shalev et al., 2012). Similarly, Drury and colleagues published a longitudinal study on 136 children living in institutions where buccal swabs were collected between 6 and 30 months of age, and compared with BTL from 54 months of age (Drury et al., 2012). In their results, percent time spent in the institution was significantly and negatively associated with BTL and remained so, even after controlling for potential cofounders like gender, ethnicity, low birth weight and age at telomere collection.

Overall, these studies consistently indicate that both ACEs and PTSD status are associated with shorter TL. However, we do not know if ACEs and PTSD status exert an influence on BTL over the entire lifespan into old age. Kiecolt-Glaser and colleagues found significantly shorter LTL and heightened inflammatory markers in a community sample of *n* =132 subjects (mean age of 69.70 years, including 58 dementia caregivers and 74 non-caregivers) when participants reported some form of physical, emotional or sexual abuse during childhood (Kiecolt-Glaser et al., 2011), and Savolainen et al. (2014) found some evidence indicating that lifetime history of adverse events might be associated with late life

LTL in American samples. However, it is not yet clear if this effect is restricted to the immune system (as indexed by changes in leukocytes) or if it will be replicated in a population who experienced such early life adversity as the Verdingkinder, a European population.

Our aim was to explore BTL differences in a sample of former Swiss indentured child laborers, currently of old age and to compare them to a healthy control group. The indentured child laborers in this study experienced both parental and familial separation as well as childhood maltreatment. Both factors were reported to have a negative impact on LTL (Drury et al., 2014; Ladwig et al., 2013; Shalev et al., 2012). We examined: a) BTL in former indentured child laborers reporting childhood adversities but no PTSD symptoms compared to healthy controls; b) BTL in former indentured child laborers reporting childhood adversities and showing PTSD symptoms compared to healthy controls; and c) the association between total reported childhood adversities and BTL. Previous studies using this specific sample have shown that some former child laborers screened positive for partial and full blown PTSD, while others remained relatively unaffected in terms of PTSD psychopathology (e.g., Burri et al., 2013). Exposure to ACEs are generally substantially higher for former indentured child labors compared to a community sample of healthy controls recruited for lack of trauma exposure. Based on previous research findings, we predicted that: a) former indentured child labors would have shorter BTL compared to healthy controls; b) individuals screening positive for PTSD will have shorter BTL compared to participants without PTSD; and c) more childhood trauma exposure will be associated with shorter BTL (Ladwig et al., 2013).

Method

Sample

This study is a sub-study of a larger project that focused on former Swiss indentured child laborers (Burri et al., 2013; Kuhlman et al., 2013). Here, we focused on a subsample of *n* = 67 indentured child laborers who had buccal cells available from a total sample of 141 individuals. For the present study, participants were recruited via advertisements in local and national newspapers and magazines, and via specific indentured child laborers societies and associations. The following inclusion criteria were applied: (Swiss-)German speaking; a minimum age of 60 years; at least one experienced period of indentured child labor; and report of at least one traumatic event. In addition, a sample of *n* = 62 demographically similar controls were recruited. Criteria for controls were set similar to those of the former indentured child laborers in order to match the samples on key variables including minimum age of 60 years, Swiss-German speaking, and were raised in rural upbringing. Furthermore, controls were raised by biological parents, and have not been diagnosed with PTSD or any other psychiatric disorder. The overall study sample of included *n* = 129 subjects. Written informed consent was obtained from all participants and the study was approved by the ethical committee of the Canton of Zurich (Switzerland).

Procedure

Psychometric data for the "Verdingkind" sample were collected between 2010 and 2012. Participants were asked to respond to a set of standardized questionnaires and structured interviews, including a testing of cognitive capacities within another project. See Burri et al.

(2013) for more information. The interviews lasted between two and three hours and were conducted either at participants' homes or at the University of Zurich by trained research assistants and doctoral students. In the context of another study, conducted between 2012 and 2013, a subsample of this indentured child laborers donated buccal epithelial cells. Simultaneously, a sample of matched healthy controls was recruited and screened with the same questionnaires as the Verdingkind sample. Contrary to the Verdingkind sample, the buccal epithelial cells of the controls were collected immediately following the interview in the first semester of 2014. Hence, the two sets of buccal samples (Verdingkind vs controls) were analyzed separately due to the independent sampling.

Materials

Childhood Trauma Questionnaire– short form (CTQ-SF)

The CTQ-SF is a 28-item self-report inventory that provides brief and reliable screening for histories of abuse and neglect. It inquires about five types of maltreatment: emotional, physical, and sexual abuse, and emotional and physical neglect. The CTQ (including CTQ-SF) is one of the most widely used instruments to assess childhood maltreatment and trauma, and has been extensively cross-validated (Karos et al. 2014; Thombs et al. 2009; Bernstein et al. 2003). In the original validation study on a US community sample (n = 1,007) aged between 18 and 65 years, a Cronbach's of .91 was obtained for the total score, .85 for emotional neglect, .83 for emotional abuse, .94 for sexual abuse, .69 for physical abuse, and .58 for physical neglect (Scher, Stein, Asmundson, McCreary, & Forde, 2001). In a recent study on 565 Swiss patients with mainly anxiety disorder or depression (86%), pedophilia (3%), or sleep disorders (11%), as well as 86 psychology students, the German

questionnaire version obtained Cronbach's α of .82 for physical abuse, .83 for emotional abuse, .90 for sexual abuse, .91 for emotional neglect, and .53 for physical neglect (Karos et al., 2014). Thus, all subscales seem to have acceptable reliability, except physical neglect. Furthermore, cutoff scores for each subscale as well as for the total score have been suggested (i.e., ≥ 13 for emotional abuse, ≥ 10 for physical abuse, ≥ 8 for sexual abuse, ≥ 15 for emotional neglect, ≥ 10 for physical neglect, ≥ 56 for the total score; Bernstein and Fink 1998).

Short Screening Scale for PTSD (SSS)

The SSS is a 7-item instrument designed to screen PTSD symptomatology in trauma survivors. The 7 items – 5 avoidance and numbing items (cluster C) and two hyper arousal items (cluster D) – were taken from the extensively validated post-traumatic stress diagnostic scale. Respondents evaluate for each item how many times it occurred over the last week. All symptoms that occur for more than two times a week add up to the overall test score. The authors suggest a cutoff score of four which best balances the scales sensitivity (80%) – the ability to detect patients with PTSD –, and specificity (97% - the ability to detect patients who do not have PTSD. Furthermore, Maercker and Pielmaier (2010) suggest a category of partial PTSD when at least one of the C-cluster symptoms and one of the D-cluster symptoms occurring 2 times or more per week were present. In a study on an adult German population (Maercker, Forstmeier, Wagner, Glaesmer, & Brähler, 2008) and a Swiss population (age >65 years; Maercker & Pielmaier, 2010), the SSS reached a Cronbach's α of =.90 and =.68, respectively.

Covariates

Depressive symptoms were measured using the Geriatric Depression Scale (GDS; Yesavage & Sheikh, 1986). The 15 questionnaire items assesses the presence of depressive symptoms based on a yes-no dichotomous response scale (e.g., "Have you dropped many of your activities and interests?"). In a German sample of 43 hospitalized patients, the GDS reached a Cronbach's α of =.91 (Gauggel & Birkner, 1999). Physical and mental functioning was assessed with a twelve item version of the Short-Form Health Survey (SF-12; Ware, Kosinski, & Keller, 1996). Additional study covariates were age, sex, self-evaluated financial situation (from "poor" to "very good") and total years of education. Self-evaluated financial situation (i.e. "How would you describe your financial situation right now?") was used as a proxy for social economic status.

Buccal Telomere Length (BTL)

For BTL participants were asked to rinse their mouths with water twice for 15 seconds prior to sample collection using two to four Isohelix Buccal Swabs (Cell Projects Ltd, 2011). According to the manufacturer`s instructions, participants were assisted in the sample collection procedure by the research assistant or doctoral students. Each buccal swab was rubbed firmly against the inside of both cheeks for 30 to 60 seconds (Freeman et al., 2003; McMichael et al., 2009). The collected buccal epithelial cells were stored with DNA stabilizer until further preparation. The insertion of a Dri-Capsule (Cell Projects Ltd, 2011) allowed the sample to be stored at room temperature without DNA degeneration (Cell Projects Ltd, 2011). Buccal cell samples were then sent to North America and analyzed by DNA Genotek (Ottawa, Canada) using quantitative polymerase chain reactions (qPCR) to assess BTL (Cawthon, 2002; O'Callaghan, Dhillon, Thomas, & Fenech, 2008). TL was determined using qPCR on a Life Technologies 7900 HT real-time instrument and Life Technologies SDS v2.4

software to estimate absolute telomere length. Primers were obtained from Integrated DNA Technologies. Briefly, the protocol described by O'Callaghan et al. (2008) uses an oligomer standard containing 14 TTAGGG telomeric repeats and a standard curve using a single copy gene standard (*36B4*) to estimate both the mean telomere length per reaction and the mean diploid genome copies for each sample. The telomere length per diploid genome and the length per telomere are then calculated according to methods described by O'Callaghan and colleagues (2008). All measurements were repeated in triplicate and mean results accepted only if the standard deviation of the cycle threshold (C_t) was < 1 C_t. Approximately 90% of samples typically pass this quality check (QC) parameter. A sample failed the analysis if it did not pass QC values for the telomere or the single-copy gene assay or both.

Statistical Analyses

To test whether participating indentured child laborers differed significantly from the rest of the sample, Welch's two sample *t*-test was conducted for continuous variables and chi-squared tests for categorical variables. Pearson correlations were computed to assess the association between BTL and the other continuous measures used in our study

Our primary hypothesis was tested using analysis of covariance (ANCOVA) by comparing BTL differences between the former indentured child laborers who screened positively or negatively for full or (partial) PTSD symptomatology and the healthy controls. In these analyses, sex, age, years of education, self-evaluated financial situation and GDS scores were adjusted. Posthoc group comparisons were conducted with the Tukey's honest significance test (HSD) to account for the differences in the group sizes (Yandell, 1997).

Since the CTQ yields continuous scores, the two samples (indentured child laborers and healthy controls) were pooled together and a stepwise hierarchical linear regression analysis was performed in three steps with BTL as the outcome variables and CTQ total scores as a predictor. The first step contained the raw CTQ total scores as a predictor. For the second model, we included age as a covariate, since TL is associated with chronological aging. In a third step we added all of the mentioned covariates (i.e. age, sex, years of education, self-evaluated financial situation and GDS scores) to the model.

Four controls were excluded from the analyses due to technical failure of the BTL assay. In order to account for leverage of statistical outliers, outliers were removed according to their Cook's distance (Aguinis, Gottfredson, & Joo, 2013). Based on a simple model where group-status predicted BTL, a Cook's distance was computed for every single case. The cutoff was set to $4/n$ (Van der Meer, Te Grotenhuis, & Pelzer, 2010). Six participants were identified as influential outliers based on Cook's distance scores and therefore removed from further analyses. After data cleaning including removal of the outliers, visual inspection and Shapiro-Wilks test ($W = 0.98$, $p = 0.18$; Royston, 1982) suggested a better approximation of the BTL measurers to normal distribution. Data handling and all analyses were conducted with R (R Foundation for Statistical Computing, 2013).

Results

Sample Characteristics

First, we checked whether the indentured child laborers who donated buccal swabs were markedly different from the original sample. Apart from marginally higher levels of emotional- (t[135.24] = 1.93, p = .06) and physical abuse (t[132.79] = 1.92, p = .06) on the CTQ, the participating indentured child laborers did not differ significantly from the rest of the (non-participating) indentured child laborers sample (all p > .16; see Table 2).

The total sample for the present analyses comprised 120 participants, including 62 indentured child laborers and 58 controls. Table 3 provides an overview of the samples' characteristics. There was no significant difference in gender between the two samples (χ2 [1, N =120] = 0.51, p > .10). The former indentured child laborers were significantly younger than the healthy controls (t[119] = 2.15, p < .001) and further reported significantly fewer years of education (t[119] = -5.36, p < .001), as well as marginally lower financial status (χ2[3, N =120] = 8.60, p = .03). A significant mean difference in years of education is to be expected, since many indentured child laborers were hindered from attaining an education (Leuenberger and Seglias, 2008). Furthermore, the two samples differed in mental (t[119] = -6.13, p < .001) and physical functioning (t[119] = -4.51, p< .001). As expected, indentured child laborers also showed significantly higher total and subscale CTQ scores (all t[119] >4.85, p < .001).

Table 2. *Sample characteristics of participating Verdingkinder and non-participating Verdingkinder*

	Participating Verdingkinder	**Non-participating Verdingkinder**	
	***M* (*SD*) / *n* (%)**	***M* (*SD*) / *n* (%)**	***p*-value**
	***n* = 67**	***n* = 74**	
Demographics			
Females [b]	30 (44.78)	28 (37.84)	.51
Age [a]	76.33 (6.13)	77.76 (7.34)	.21
Years of education [a]	10.47 (2.15)	10.22 (3.32)	.57
Self-evaluated financial situation [b]			.35
Poor	6 (8.96)	13 (17.57)	
Fair	17 (25.37)	22 (29.73)	
Good	28 (41.79)	25 (33.78)	
Very good	15 (22.39)	13 (17.57)	
Marital Status [b]			.87
Single	3 (4.05)	5 (6.76)	
Married	28 (41.79)	28 (37.84)	
Separated or divorced	17 (25.37)	17 (22.97)	
Widowed	19 (28.36)	24 (32.43)	
GDS scores [a]	3.17 (3.01)	3.88 (4.01)	.26
SF-12: Physical functioning [a]	42.86 (11.55)	42.16 (11.72)	.72
SF-12: Mental functioning [a]	48.31 (10.29)	49.25 (10.71)	.60
CTQ scores			
CTQ total score [a]	74.52 (20.42)	70.00 (17.01)	.16
Emotional abuse [a]	15.27 (6.14)	13.32 (5.77)	.06
Physical abuse [a]	14.00 (6.99)	11.85 (6.22)	.06
Sexual abuse [a]	9.90 (6.59)	8.92 (5.77)	.35
Emotional neglect [a]	20.40 (5.17)	21.09 (5.05)	.41
Physical neglect [a]	14.97 (4.03)	15.29 (3.64)	.63

Note. Two participant of each Verdingkinder group refused to disclose information about their self-evaluated financial situation. [a] Welch's two-sample *t*-test; [b] χ^2-test

Table 3. *Sample characteristics of Verdingkinder and controls*

	Verdingkinder	**Controls**	
	***M* (*SD*) / *n* (%)**	***M* (*SD*) / *n* (%)**	***p*-value**
	***n* = 62**	***n* = 58**	
Demographics			
Females [b]	27 (43.55)	30 (44.11)	.48
Age [a]	76.19 (6.18)	71.85 (5.97)	<.001
Years of education [a]	10.45 (2.16)	13.35 (3.57)	<.001
Self-evaluated finical situation [b]			.04
Poor	5 (8.06)	0 (0.00)	
Fair	16 (25.81)	10 (17.24)	
Good	26 (41.94)	37 (63.79)	
Very good	14 (22.58)	11 (18.97)	
Marital Status [b]			.33
Single	2 (3.23)	3 (5.17)	
Married	25 (40.32)	23 (39.66)	
Separated or divorced	17 (27.42)	11 (18.97)	
Widowed	18 (29.03)	12 (20.69)	
GDS scores [a]	3.23 (3.10)	0.45 (0.80)	.23
SF-12: Physical functioning [a]	42.71 (11.91)	50.64 (6.88)	<.001
SF-12: Mental functioning [a]	48.44 (10.37)	56.82 (2.80)	<.001
CTQ scores			
CTQ total score [a]	73.27 (20.17)	34.31 (7.77)	<.001
Emotional abuse [a]	14.98 (6.18)	6.60 (2.38)	<.001
Physical abuse [a]	13.58 (6.80)	5.76 (1.90)	<.001
Sexual abuse [a]	9.66 (6.45)	5.57 (1.51)	<.001
Emotional neglect [a]	20.12 (5.27)	9.45 (4.21)	<.001
Physical neglect [a]	14.92 (4.14)	6.93 (2.25)	<.001

Note. Sample sizes are indicated for samples after exclusion of outliers. [a] Welch's two-sample *t*-test; [b] χ^2-test

Forty-eight of the 62 (77.42%) former indentured child laborers reported CTQ total scores high enough to indicate moderate to severe childhood trauma, while the scores ranged from 32 to 115. The range for CTQ scores in the 58 controls spanned from 25 to 56, with only one control scoring at the clinically significant cutoff of 56 and all others scored 53 or lower. Nine former indentured child laborers screened positive for partial PTSD and 12 for full PTSD. For subsequent analyses, individuals with partial and full PTSD were combined into one group, resulting in 21 individuals reporting previous moderate to severe childhood trauma and PTSD, 41 participants with moderate to severe childhood trauma but no PTSD, and 58 controls reporting neither previous moderate to severe childhood trauma nor PTSD.

PTSD and BTL

In order to test our hypothesis about the relationship between PTSD symptom status and BTL, analysis of covariance including three factors (*Controls* vs. *PTSD negative* vs. *PTSD positive*) were conducted by simultaneously controlling for the effects of age, sex, years of education GDS score, mental-, and physical functioning. Analyses indicated that PTSD symptomatology was significantly associated with BTL, $F(2,109) = 3.27$, $p = .04$, $\eta^2 = 0.06$ in these analyses. Tukey's HSD indicated that healthy controls showed significantly ($p = .04$) shorter BTL ($n = 58$, $M = 4.30$, SD =1.73) compared to the PTSD positive former indentured child laborers ($n = 21$, $M = 5.55$, SD =1.90), resulting in a BTL mean difference of 1.25 (95%CI: 0.04 – 2.46). No significant differences between the PTSD negative former indentured child laborer group and the PTSD positive former indentured child laborer group ($n = 41$, $M = 4.74$, SD =2.38), or between the PTSD negative former indentured child

laborer group and the control group were detected (both $p < .29$, resulting in a BTL mean difference of 0.80 [95%CI: -0.46 – 2.08] and 0.44 [95%CI: -0.53 – 1.41] respectively). Figure 4 A provides a visualization of the results.

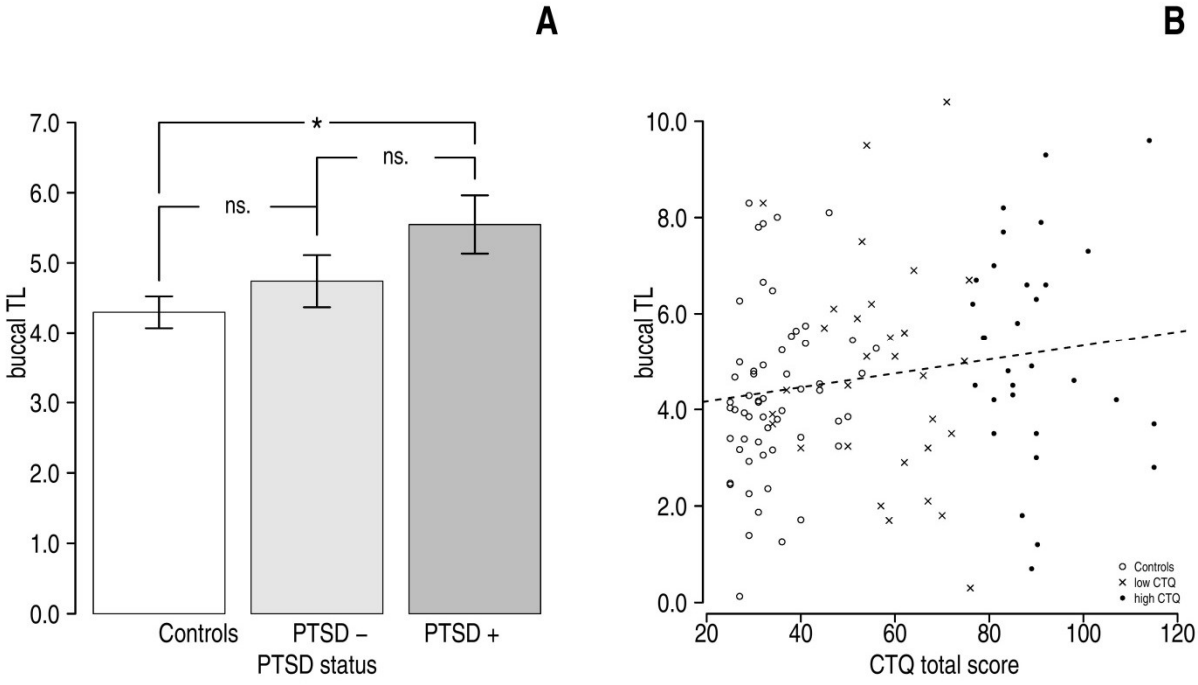

Figure 4. *Buccal telomere length differences by groups.*

Note. **A.** Analysis of covariance indicated that there was a significant effect of post-traumatic stress disorder (PTSD)-status ($F(2,109) = 3.27$, $p = .04$; $\eta^2 = 0.06$). BTL tended to increase from controls ($n = 58$, $M = 4.30$, $SD = 1.73$) to former indentured child laborers with PTSD ($n = 21$, $M = 5.55$, $SD = 1.90$), whereby former indentured child laborers with PTSD negative symptomatology ($n = 41$, $M = 4.74$, $SD = 2.38$) did not demonstrate significantly shorter BTL than either indentured child laborers with PTSD positive status or controls (both $p > .292$)**. B.** Scatterplot illustrating the relationship between unadjusted BTL and childhood trauma questionnaire total scores. Higher childhood trauma scores were associated with longer mean BTL.

ACEs and BTL

Correlation analyses indicated that BTL was only marginally associated with CTQ scores and not associated with age or the other measures in the pooled sample. Furthermore, CTQ was associated with all measures, except the participants' age. Table 4 gives an overview of these correlations results.

Table 4. *Correlations between BTL and the continuous covariates*

Measures	***1.***	***2.***	***3.***	***4.***	***5.***	***6.***
1. BTL	–					
2. CTQ total score	0.18‡	–				
3. Age	0.06	0.08	–			
4. Years of education	-0.09	-0.39**	-0.35**	–		
GDS score	0.09	0.51**	0.29**	-0.35**	–	
SF-12: Mental functioning	-0.06	-0.49**	-0.12	0.29**	-0.69**	–
SF-12: Physical functioning	-0.01	-0.34**	-0.26**	0.14	-0.42**	0.23*

Note. Correlations were conducted over the sample of n = 120 participants that enrolled the main analyses.
*‡ p > .10; *p > .05; ** p > .01*

To test whether BTL was associated with CTQ scores, we performed a hierarchical linear regression analysis. First, only CTQ scores were entered into the model, resulting in a marginally significant effect showing higher CTQ scores to be associated with longer BTL (β = 0.18 [95% CI: 0.00 – 0.35], t = 1.92, p = .05; see Figure 4 B. Since BTL is associated with biological aging, a second model adjusted for age was computed. In this model, age was not

associated with BTL (β = 0.05 [95% CI: -0.13 – 0.23], t = 0.53, p = .60), consequently the association between the CTQ total score and BTL remained marginally significant (β = 0.17 [95% CI: -0.01 – 0.35], t = 1.90, p = .06). In a third and final step the remaining covariates sex, years of education, self-evaluated financial situation, GDS score, mental-, and physical functioning were included in the model. The full adjusted effect size of the CTQ total score was slightly larger but remained only marginally significant (β = 0.25 [95% CI: -0.01 – 0.49], t = 1.64, p = .06).

Within group associations between trauma and BTL

In exploratory analyses, we examined relationships between CTQ and BTL within the two subsamples of former indentured child laborers (n = 62) and the controls (n = 58). As with the full sample analysis, we computed three different regression models for both samples. In the first model we only predicted BTL with CTQ total scores; in the second model we adjusted for age and in the third model we adjusted for all covariates (i.e. sex, years of education, self-evaluated financial situation and GDS scores.).

In these models, CTQ total score did not predict BTL significantly, neither within the former indentured child laborer sample (β = 0.03, t = 0.23, p = .08), nor the control sample (β = 0.20, t = 1.52, p = .14). Adjusting for age (former indentured child laborer sample: β = 0.10, t = 0.68, p = .50; control sample: β = 0.18, t = 1.40, p = .17) or adjusting for the remaining covariates (former indentured child laborer sample: β = 0.12, t = 0.62, p = .46; control sample: β = 0.21, t = 1.50, p = .14) did not change the results significantly.

Discussion

The aim of this study was to assess the association between childhood trauma, PTSD and BTL in a sample of elderly formerly indentured Swiss child laborers (i.e., "Verdingkinder"). Contrary to our hypotheses, we were unable to observe shorter mean BTL in the sample of former child laborers compared to a group of healthy control subjects. Instead, former indentured child laborers screening positive for partial or full PTSD symptomatology showed longer BTL compared to healthy controls; this effect remained significant even after controlling for age, sex, years of education and depression. The differences between the healthy controls and indentured child laborers without no significant PTSD symptoms, as well as the difference between indentured child laborers with PTSD compared to without PTSD were both statistically non-significant (See Figure 4 A). We also examined if self-reported childhood trauma was associated with BTL. Again – contrary to our hypotheses – we found no significant associations and in fact, a tendency for higher CTQ scores to be associated with longer age-adjusted BTL. Thus, we failed to replicate previous findings of shorter TL associated with PTSD and child trauma exposure. Explanations include possible resilience in our group of surviving elderly maltreated indentured child laborers and a lack of persistence of the effects of child trauma into late life.

To our knowledge, this is the first study to examine the association between PTSD symptomatology, ACEs and BTL in a sample of elderly people. The indentured child laborers in our sample experienced both parental and familial separation as well as high rates of maltreatment. Prior research shows that parental separation in itself is a form of early adversity that can have strong negative impact on mental and physical health (Ladwig et al., 2013). Contrary to our hypotheses and in contrast with some studies (Kiecolt-Glaser et al.,

2011; Ladwig et al., 2013; Savolainen et al., 2014; Idan Shalev et al., 2012), but in line with recent findings (Schaakxs et al., 2015), shorter mean BTL was observed in controls compared to indentured child laborers with PTSD. Prior studies in older adults have assessed LTL instead of BTL (Kiecolt-Glaser et al., 2011; Ladwig et al., 2013; Savolainen et al., 2014). Given that our results are inconsistent with these prior studies, it is also possible that the effects of early life adversity on cellular aging are not evident in buccal cells.

Alternatively, the finding of longer BTL in traumatized individuals with PTSD symptomatology may suggest a resilience hypothesis. Given the known adverse effects of psychological stress on biological age and mortality, it is possible that less healthy and less resilient individuals from the indentured child laborer population died earlier and never had the chance to participate in this study in the first place. There is currently no specific study that reports on mortality in former indentured child laborers, but other studies do suggest that traumatic stress may lead to early mortality (Boscarino, 2006, 2008). It is therefore possible that those individuals who did participate could be described as a potentially unique selection of resilient survivors who, despite their negative psychological experiences, did not necessarily undergo accelerated biological aging.

In opposition to this hypothesis are the high rates of PTSD symptom scores observed in our sample of indentured child laborers. If our sample is truly resilient, it is surprising that they demonstrate such high levels of trauma-related psychopathology. One explanation for these high levels of trauma-related psychopathology might be found in political issues within Switzerland. Over the last few years, there has been a national debate in Switzerland over the compensation of peoples who have been indentured. It is therefore possible that these factors influenced the participants' responses such that some participants hoped to increase their chances for compensation when they reported more childhood trauma and

demonstrated PTSD symptoms. Such biases may be conscious or unconscious, as indicated by studies of US military veterans (McNally & Frueh, 2013).

We further tested our post-hoc assumption that our participants might constitute a sample of particularly resilient subjects by dividing the sample into a group with longer mean BTL and a group with shorter mean BTL by a median split. These analyses did not reveal any significant associations between BTL and any of the variables, including gender, mental-, or physical functioning, familial status, financial status or depression symptomatology in any of the two groups. Therefore, the assumption that the surviving elderly indentured child laborers were resilient was not supported. Nevertheless, it is possible that our sample were physiologically resilient to stress, even if not psychologically resilient. Unfortunately, our design did not allow us to control for survivor effects and thus this hypothesis must be addressed in a future prospective longitudinal design. And like indicated in the introduction section, there is unfortunately also no public record (e.g. vital records) that could further explore the mortality rate and resiliency of former indentured child laborers in Switzerland and compare it to that of the general Swiss population. Nevertheless, similar findings were reported in another trauma survivor study (Sagi-Schwartz, Bakermans-kranenburg, Linn, & Ijzendoorn, 2013).

Lastly, this finding has to be interpreted with caution. First, TL research (Ladwig et al. 2013; Kiecolt-Glaser et al. 2011; Savolainen et al. 2014) has predominantly focused on TL derived from leukocyte populations or, in cases where buccal epithelial cells were used, the analyses were conducted in younger samples (Shalev et al. 2012). It has not yet clearly been established how BTL behaves as a function of age, and whether the results can be extrapolated to other tissue samples, such as leukocyte samples, since buccal epithelial cells are exposed to a number of exogenous stressors and have a very prolific nature. So far,

stability between different tissue samples remains an ongoing field of research. Only a few studies have compared TL concordance across different tissues (Friedrich et al., 2000; Kimura et al., 2010; Nakamura et al., 2002; Okuda, 2002; Thomas et al., 2008; Youngren et al., 1998). To our knowledge there is currently no study that observed TL dynamics across multiple time points from different tissue samples. Nevertheless, some cross-sectional data indicate that there may be substantial synchrony across different tissue samples. For example, did Friedlich and colleagues find that the associations between epidermis TL, LTL and synovial TL samples were very high in a small sample of 9 patients (Friedrich et al. 2000). And in two post-mortem samples with 41 and 21 subjects (aged 0 to 101 years), the correlation between lingual epithelium cell TL and epidermis TL was with r = .84 and r = .93 also very high (Nakamura et al. 2002). Other studies found no significant correlation between BTL and whole blood cell TL in samples with 110 subjects from 18 to 93 years of age (O'Callaghan et al. 2008). Furthermore, while there are several indications that there is a substantial correlation between TL in different tissues, it is important to note that most studies on the subject of tissue-specific TL correlations incorporated only small sample sizes.

Limitations

This study has some limitations. First, participants were fully aware of their status as indentured child laborers or healthy controls, due to recruitment criteria. For example, healthy controls were excluded when they exhibited psychopathology or when they grew up in problematic households. It is plausible that this recruitment procedure had an indirect effect on the self-reporting tendencies of the participants. Self-reported

psychometric data always holds the danger of recall bias. Hence, information about childhood experiences might have been positively (in the case of the control group) or negatively (in the case of the indentured child laborers) biased. Apart from psycho-symptomatology, the groups did also differ in terms of some sociodemographic variables, including age and education.

Second, in order to assess current PTSD symptomatology, the PTSD SSS was used. Despite the instrument's validity, the SSS can only be regarded as a screening instrument for PTSD symptoms and does not provide clinical diagnosis for PTSD. Third, given the unique sample of former indentured Swiss child laborers, it is not surprising that only few former Verdingkinder participated and also demonstrated current PTSD symptomatology, hence, resulting in a relatively small sample size. Therefore, future studies should aim at incorporating larger samples.

Furthermore, our study had a small sample size, which ultimately limited the statistical power of our study. Sensitivity power analyses indicated that our sample was large enough to detect only medium to large effects. It is therefore worth noting that our study may have lacked sufficient power to detect the effects at hand. However, it is worth noting that the observations in our study went in the opposite direction of our hypotheses. It is therefore plausible that our null findings are not due to a lack of statistical power, but rather based on the absence of assumed accelerated cellular ageing processes in the collected buccal samples.

Lastly, our analyses suffer from a potential batch effect (Soneson, Gerster, & Delorenzi, 2014). Due to the fact that the control sample was only recruited after the initial indentured child laborers sample, we were not able to simultaneously process both batches. Even though the qPCR-protocol is reliable and precautions were taken to ensure validity of

the results (O'Callaghan et al., 2008), a potential batch bias is possible. However, even within-group analysis did not indicate confirmation for our general hypothesis (in neither the former child laborer sample nor the control sample), indicating that a lack of a negative association between childhood trauma and BTL cannot be attributed to a batch effect alone. Moreover, higher CTQ scores tended to be associated with longer BTL within the indentured child laborer sample as well as within the full sample. It is also worth noting, that secondary analyses revealed no association between the time that passed between BTL and time elapsed between collection of the buccal swabs and the qPCR, indicating that duration of storage did not affect the results. Nevertheless, this methodological issue limits the confidence we can have in our group comparison; however, it is important to note that we also did not support our hypothesis that childhood trauma would be associated with shorter BTL within former indentured child laborers or controls either.

Conclusion

In conclusion, this is the first study to extend our knowledge about the association between childhood adversity, PTSD and TL into old age. We were unable to confirm our hypothesis that ACEs and PTSD symptomatology contribute to BTL in later life. Our study results hold the possibility of a resilient-survivor-phenotype amongst populations exposed to severe childhood stress, even though we were not capable of addressing or confirm this alternative assumption in this particular study. Further studies are needed to address this question in a prospective longitudinal manner to account for survivor effects. Given the nature of our findings and lack of other studies on the long-term consequences of childhood adversity on TL biology, the field of stress and telomere biology should focus more on examining resilience in trauma survivors.

7 Paper Three: Transgenerational Aspects of Former Swiss Child Laborers: Do Second Generations Suffer From Their Parents' Adverse Early-Life Experiences?

(Küffer, Thoma, & Maercker, 2016)

Abstract

Background: Recent research suggests that childhood adversity not only exerts a lasting impact on the affected individuals but also on their offspring. Little is known about the role of parental rearing behavior on the transgenerational conveyance of parental childhood adversity on filial psychological health.

Objective: It was therefore the aim of the current study to investigate the relation between parental rearing behavior of former Swiss indentured child laborers ("Verdingkinder") and psychological health of their adult offspring.

Methods: We applied a two-generation control-group design with two parental samples (n = 16, former "Verdingkinder", M_{age} = 76.13, SD = 6.81 and n = 19, parental controls, M_{age} = 72.63, SD = 5.96) and their offspring (n = 21, former "Verdingkinder" offspring, M_{age} = 52.91, SD = 5.90, and n = 29 offspring controls, M_{age} = 44.55, SD = 7.71) were examined. Parental rearing behavior, childhood trauma, and psychological health were assessed with questionnaires. Data were analyzed using Bayesian analyses, where Bayes factors (BF) of 3 or higher were considered as substantial evidence for the tested hypotheses.

Results: We found that “Verdingkinder” offspring reported more physical abuse (BF_{10} = 5.197) and higher total childhood trauma exposure (BF_{10} = 2.476). They described both their fathers (BF_{10} = 14.246) and mothers (BF_{10} = 24.153) as less emotional and their mothers as more punitive (BF_{10} = 18.725). An increased sense of reflection, e.g. ones ability to take different perspectives, was found in the offspring controls (BF_{10} = 5.245). Furthermore, exploratory analyses revealed that perceived familial emotionality was associated with higher psychopathology (all BF_{10} = 10.471) and higher pessimism (all BF_{10} = 5.396).

Discussion: Our data provides cross-sectional evidence of a meaningful transgenerational relation between parental childhood adversity, dysfunctional rearing behavior, and psychological health of their offspring. Prospective studies are needed to investigate these findings in a longitudinal setting.

Keywords: Childhood maltreatment, transgenerational effect, psychopathology, parental rearing behavior, sense of coherence, pessimism/optimism

Introduction

Early life adversity, such as trauma or maltreatment can exert a "long shadow" (Brent & Silverstein, 2013), i.e. profound and, thus, long-lasting impact on the affected individual (Liu & Umberson, 2015; Springer et al., 2003; Umberson et al., 2005). The World Health Organization (WHO), who conducted a world-wide survey including over 50'000 respondents concluded that childhood adversities are strongly associated with adult mental disorders and are found to predict 29.8% of DSM-IV disorders (Kessler et al., 2010). Stressful experiences in childhood are, thus, a strong predictor of adult mental health.

The impact of early-life adversity may go beyond the lifetime of the affected individual and spread to the next generation. In a study with Cambodian students, parental trauma symptomatology due to surviving the Khmer Rouge genocide as a child or adolescent was associated with anxiety and depression in offspring (Field et al., 2011). In another study with Rwandan mothers who survived the genocide of 1994, it was found that maternal childhood violence exposure was associated with offspring anxiety, depression, and behavioral disorders (Roth et al., 2014). Together, existing data derived from transgenerational research suggest that parentally experienced early life-adversity exert a meaningful impact on the next generation's mental health.

Limited information exists with regard to the underlying mechanisms. One potential process responsible for the transgenerational conveyance of parentally experienced early-life adversity on offspring's mental health could be parenting style. On one hand, parenting style has repeatedly been found to be associated with offspring psychopathology, such as depression and anxiety (Lima et al., 2010), schizophrenia (Skagerlind, Perris, & Eisemann, 1996), personality disorders (Giakoumaki et al., 2013), and eating disorders (Tetley et al.,

2014). On the other hand, parenting style has also been related to adverse childhood experiences. For instance, a group comparison between adult offspring of 159 Holocaust survivors and 151 controls revealed that Holocaust offspring experienced more parental punishment than controls (Kellermann, 2001). Similarly, in a Spanish sample with over one hundred female hospital patients, it was found that perceived parental rearing style, i.e. coldness, detachment, rejection and parental overprotection, was related to self-reported childhood abuse and neglect (Hernandez et al., 2013). Similar results have been reported in a laboratory study of German mothers with self-reported early-life abuse and their 5-month-old infants. Mothers with a history of abuse were found to be more intrusive toward their infants as compared to mothers with no history of abuse (Moehler, Biringen, & Poustka, 2007). Taken together, existing data point toward a meaningful relationship between parental style and offspring's mental health as well as early-life adversity and parental rearing behavior.

Only a few studies exist that have looked at the mediating effect of parental rearing behavior on the relationship between parentally experienced early-life adversity and offspring mental health. In a series of investigations with Cambodian participants conducted by Field and colleagues, it was found that the association between parental trauma and offspring psychopathology was mediated by parenting style, i.e. parent's role reversing and maternal overprotection (Field et al., 2011). However, conclusions are limited, as findings rely on self-reports of the teenage offspring only. In 2013, the authors conducted another two studies, this time including the Cambodian mothers, too. The authors were able to extend previous findings by showing that the parenting style of the mothers mediated the impact of maternal PTSD symptomatology on offspring's anxiety and depression (Field et al., 2013). The authors were not only able to find the trauma

transmission via parenting style in Cambodian dyads but also in Cambodian-American refugee dyads. While these valuable studies point toward a meaningful moderator / mediator effect of parental rearing behavior on the relationship between parentally experienced childhood adversities and offspring's psychopathology, generalization of these results to parenting samples of Western origin involving more minor trauma is limited. Also, particular focus was put on mothers, restricting conclusions to mother – child pairs. Finally, hardly anything is known about the influence of parentally experienced early-life adversity on the offspring's resilience. A worthwhile exception is typified by a recently published study conducted with adult children of Holocaust survivors that included indicators of resilience, such as sense of coherence (Fossion et al., 2014). In this study, it was shown that adult offspring from less functional families reported a lower sense of coherence when compared to the general population, suggesting less resources to cope with stress (Fossion et al., 2014). However, it remains unclear whether this holds true only for the offspring of parents surviving genocide.

It was, therefore, the aim of the current study to examine the role of parental rearing behavior in the transgenerational transmission of parentally experienced early-adversity on psychological health of Verding-children's offspring. For this, we examined a historically unique sample of male and female former Swiss indentured child laborers, the so-called "Verdingkinder". Until the late 1970s, it was a common practice in Switzerland to remove orphans, children of single-parent families, or even children from divorced or separated parents from their family environment and place them into indentured child labor. Children were mainly sent into farmers' homes to work for their living. Historiographical studies have documented the harsh environment in which these individuals grew up, reporting that a large proportion of the children were regularly beaten, emotionally and sexually abused,

and that some were even beaten to death (Furrer, Heiniger, Huonker, Jenzer, & Praz, 2014; Leuenberger & Seglias, 2008). Survivors of the former "Verdingkinder" are now in late life, and studies have reported high prevalence of adverse childhood experiences and poor mental health (Burri et al., 2013; Maercker et al., 2015; Simmen-Janevska et al., 2014). Even though childhood trauma is not necessarily associated with former childhood labor, the sample of former "Verdingkinder" reported here was specifically selected for their childhood trauma in order to represent a common form of the "Verdingkind" phenomenon (Leuenberger & Seglias, 2008).

Our particular research questions were the following: Is there evidence for higher adverse childhood experiences in the offspring generation of parents that grew up at high risk of experiencing adversities and trauma compared to a control offspring generation? Is there evidence for dysfunctional parental rearing behavior in families of former "Verdingkinder"? Are there differences between the offspring samples with regard to psychopathology, and psychological health? Furthermore, exploratory analyses investigated the interactions of parental rearing behavior and family type ("indentured child labor family" vs. "control family") associated with offspring psychopathology and psychological health.

Methods

Sample and recruitment

Data for the parental sub-sample of the former "Verdingkinder" has been collected within a larger study on long-term consequences of indentured child labor in Switzerland conducted by our research group between 2010 and 2012 (Burri et al., 2013; Kuhlman et al., 2013; Maercker et al., 2015). Within a project on biological consequences of their childhood experiences and trauma, a sub-sample of former child laborers provided further information (Küffer, O'Donovan, Burri, & Maercker, 2016). Data collection for the parental control sample was done within the context of the current study between 2013 and 2014. We expanded these samples by their offspring.

Former indentured child laborers were recruited via advertisements in local and national newspapers and magazines, and via particular associations and societies for former "Verdingkinder". For the sub-sample of former "Verdingkinder" following inclusion criteria were applied: (Swiss-)German speaking, a minimum age of 60 years, at least one experienced period as indentured child laborer, and a report of at least one traumatic event in their life-time. The parental control sub-sample was recruited via mouth-to-mouth propaganda and mailing-lists of the University of Zurich. Inclusion criteria included a minimum age of 60 years, (Swiss-) German speaking, upbringing in rural communities or on the countryside, having been raised by their biological parents, and being free of any psychiatric diagnosis including PTSD. In order to be able to establish contact with the offspring of the parental sub-samples, all participants with children were contacted in written form and asked to inform their children about the current study. Parental participants' offspring were contacted by research-coordinators via phone or email only if

they showed interest in the study. All contacted children that expressed interest in participation were then provided with written study-information material (see Figure 5). Written informed consent was obtained from all participants (parents and children) and the study-procedure was approved by the ethical committee of the Canton of Zurich, Switzerland (KEK-ZH-Nr. 2012-0245).

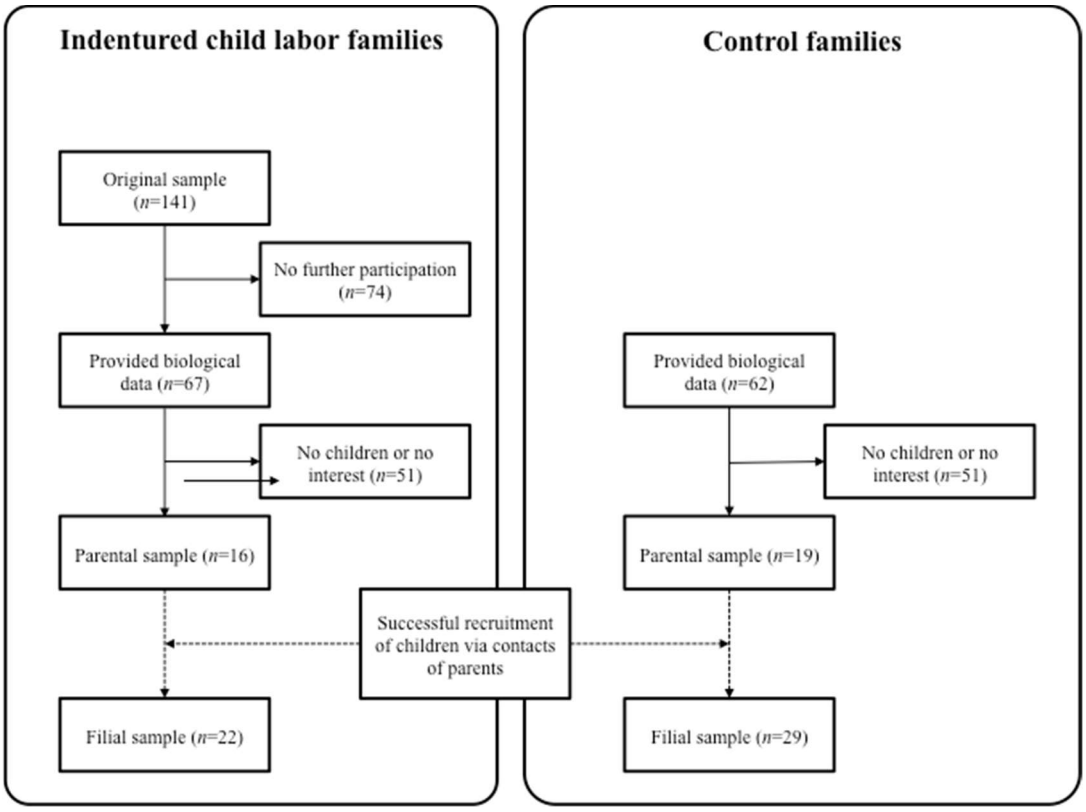

Figure 5. *Flowchart of sample recruitment procedure.*

Procedure

Specifically trained research assistants and graduate students interviewed the parental sub-samples in one-to-one interviews. The interviews lasted between two to three hours, including refreshment breaks, and were either conducted at the University of Zurich or at the participants' home, depending on the preference of the participants.

Data assessment of the offspring sub-samples was achieved through paper and pencil questionnaires, which were sent to their homes by mail. A franked envelope was included with the questionnaire package. The completion of the questionnaire lasted approximately 30 minutes. Participants received no financial incentives. However, the parental sub-samples were reimbursed for their travel expenses in case the interview was held at the Psychological Institute of the University of Zurich.

Instruments

The following instruments were used to assess parental and filial trauma exposure, recalled parental rearing behavior (from an offspring perspective), offspring psychopathology, offspring optimism / pessimism, and sense of coherence:

Childhood Trauma Questionnaire– short form (CTQ-SF, Bernstein et al., 2003): The CTQ-SF is a 28-item self-report inventory that provides brief and reliable screening for histories of abuse and neglect. It inquires about five types of maltreatment: emotional, physical, and sexual abuse, and emotional and physical neglect. The CTQ (including CTQ-SF) is one of the most widely used instruments to assess childhood maltreatment and trauma, and has been extensively cross-validated (e.g., Karos et al., 2014). In the original validation study on a US community sample (n = 1'007) aged between 18 and 65 years, Cronbach's α reached was

satisfying for the physical subscales (> .58) to good in the case of emotional and sexual subscales (> .83) (Scher et al., 2001).

Questionnaire of Recalled Parental Rearing Behavior (QRPRB, Schumacher et al., 1999): The QRPRB is a 2x24 item German questionnaire for adult participants that assess recalled maternal and paternal behavior in the dimensions of rejection and punishment, emotional warmth, and control and overprotection. Each subscale has eight items that are summed up. The QRPRB has been validated in a sample of *N* = 2968 participants and reviled internal consistency of Cronbach's α = .72 – 89.

Brief Symptom Inventory (BSI, Derogatis and Spencer, 1993): The BSI consists of 53 items that assess nine psychological symptom dimensions somatization, obsession-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism. For the current analysis, the global severity index (GSI) was used. The GSI is a sum-score of all nine dimensions to indicate a participants' global, psychological distress. Cronbach's α for the nine subscales and the GSI was reported to be higher than .70 (Boulet and Boss, 1991).

Revised Life Orientation Test (LOT-R, Scheier et al., 1994): Within the filial sample, optimism and pessimism were measured using the LOT-R (Scheier et al., 1994). The instrument comprises three positively worded items, which comprise the optimism subscale (e.g., "In uncertain times, I usually expect the best"); three negatively worded items, which comprise the pessimism subscale (e.g., "If something can go wrong for me, it will"); and four filler items. Participants are asked to rate their agreement or disagreement with each of the statements on a five-point scale from 0 ("strongly disagree") to 4 ("strongly agree"). Internal consistency for the optimism (Cronbach's α = .88) and pessimism (Cronbach's α = .78) subscales was high.

Sense of Coherence Revised (SOC-R, Bachem & Maercker, 2016): The sense of coherence was assessed within the filial sample with SOC-R (Bachem & Maercker, 2016). The questionnaire compromises 13 items that are divided into three dimensions of *manageability*, *reflection* and *balance*. Items are assessed on a five-point scale from 0 ("not at all") to 4 ("completely"). In a sample of 60 bereaved persons, the SOC-R had a test-retest reliability of .85 over the period of four weeks and one of .75 for the period of 15 months. In the validation study of Bachem and Maercker (in press) that analyzed two different samples, the Cronbach's α reached from .57 (for balance) to .77 (for manageability). The total scale had Cronbach's α of .75 and .81.

Covariates: Additional study covariates were age, sex, self-evaluated financial situation (from "poor" to "very good") and total years of education. Self-evaluated financial situation (i.e. "How would you describe your financial situation right now?") was used as a proxy for social economic status.

Statistical Analysis

Data handling was conducted with R (R Core Team, 2013) and statistical analysis was conducted with R and JASP, an open source software for Bayesian data analyses (Love et al., 2015). Since some participants from the filial generation were siblings, the data presented here was nested within the 35 families, and therefore, theoretically non-independent. Therefore, intraclass correlation-coefficient (ICC; Bliese, 2000) was assessed for all potential dependent variables. Since all ICC were trivial (all ICC < .10), all analyses were conducted under the assumption of statistical independence.

Due to the relatively small sample sizes and the fact that not all dependent variables were normally distributed, Bayes factors (BF) were computed with Bayesian t-tests and Bayesian analyses of covariance (BANCOVA) for hypotheses testing and exploratory analyses of interactions, instead of conventional inference by null-hypotheses testing (Rouder, Morey, Speckman, & Province, 2012; Rouder, Speckman, Sun, Morey, & Iverson, 2009).

According to Van de Schoot et al. (2014), in Bayesian statistics the key difference to null-hypotheses significance testing (NHST) lies in the nature of the unknown parameters. The framework of NHST assumes that the parameters of interest are unknown, but fixed in the true population. For example, in the context of our study, one true CTQ mean could be determined for the whole population of all former indenture child laborers. The Bayesian standpoint assumes that unknown parameters are uncertain and should therefore be described by probability distributions. E.g., the mean of the CTQ score in the population of former indentured child laborers is not fixed and rather expressed in the form of a probability distribution. In order to estimate this probability distribution, Bayesian inference relies on three "ingredients" (Van de Schoot et al., 2014). The first concerns the background knowledge on the parameters a researcher gathers before model testing. This background knowledge is reflected in the prior distribution, which then comes to represent the assumptions of a researcher before analyzing the data. Furthermore, the variance of the prior distribution describes the level of uncertainty about an investigated population value. For instance, if the researcher expects no specific effect between two populations and assumes that every effect is possible but more probable for small effects (either positive or negative), the prior distribution variance is large. In this example, there is only little certainty about the population value of the expected effect. Given more information, though,

the researcher can restrict the variance of the prior distribution by using a more clearly defined effect. As an example from our study, if there was no reason to assume a specific effect between the control sample and the "Verdingkinder" sample, we did not restrict the prior distribution, while assuming that smaller effects were more probable than larger effects. On the other hand, if we had reason to expect a specific directionality for the effect, we restricted the variance of the effect in the given direction. For instance, we expected lower total CTQ scores for controls vs. former indentured child laborers. Therefore, the variance of the effect was not allowed to be negative for this analysis. Despite an increased precision for the second case with restricted variance for the expected effect, both prior distributions are fairly low in precision and are therefore referred to as low-informative prior distributions. The important step in Bayesian approaches is the distribution of the assessed data that is expressed in terms of the likelihood function (Van de Schoot et al., 2014). Via Bayes' theorem, the prior distribution and the likelihood function of the assessed data are reconciled into the posterior distribution. The posterior distribution reflects the updated knowledge of our investigated parameters and consists of prior knowledge, weighted by the evidence gathered in a given study.

One way to draw statistical inference with Bayesian approaches is the use of BFs (Andraszewicz et al., 2015). BFs grade the decisiveness of the evidence by comparing the posterior beliefs for H_0 against the posterior beliefs for H_1. With other words, a BF can be expressed as odds for support or evidence for the H_0 over H_1 (BF_{01}) or as support for H_1 over H_0 (BF_{10}). A practical guide on Bayesian approaches by Jaroz and Wiley (2014) lists verbal labels that allow a more intuitive interpretation of these BFs/odds. For instance, Wetzels et al. (2011) interpret a $BFs_{10} < 1$ as non-evidential, BFs from 1 – 3 as anecdotal or weak, BFs from 3 – 10 as substantial evidence for the H_1, BFs from 10 – 30 as strong

evidence, BFs from 30 – 100 as very strong evidence, and BFs > 100 as decisive evidence. It is important to understand that BFs represent an odds-ratio in favor or against tested hypotheses or models and those listed labels can be interpreted on a continuum, rather than on the basis of discreet thresholds (as for instance with significance levels of p-values).

Furthermore, Bayesian approaches have several distinctive advantages over NHST (Van de Schoot et al., 2014): Firstly, with the use of BFs it is possible to compare specific models and hypotheses directly (Andraszewicz et al., 2015), while NHST classically informs about the probability of some data, given the H_0 (Cohen, 1994). Therefore, Bayesian inference with the use of BFs allows for an interpretation of the evidence against any given hypothesis, thus, also against the H_0. NHST on the other hand only permits us to reject the H_0 if a *p*-value falls below the significance level, but does not facilitate an interpretation of the result in favor of the H_0 in case of a non-significant *p*-value. Furthermore, an advantage of Bayesian statistics over NHST is that Bayesian approaches work with the given data directly, rather than assuming that they behave like data that are normally distributed. In the case of non-normal distributed parameters, Bayesian analyses provide more accurate results and allow statistical inference even in absence of normality (Van de Schoot et al., 2014). Bayesian approaches are also well-suited to deal with small sample sizes. Even though the posterior distribution is more affected by the choice of the prior distribution if the sample size is smaller (due to fewer evidence) (Van de Schoot et al., 2014), Bayesian inference remains a better choice for small sample sizes than NHST. This seems to be also the case if default prior settings were chosen, as it was the case in our analyses.

See Van de Schoot and colleagues (2014) and/or Andraszewicz and collegues (2015) for a more detailed introduction to Bayesian inference.

JASP allows us to specify the direction of group difference one expects in group comparisons, by restricting the prior distribution of the effect size – in this case a default Cauchy prior = 0.707 – to be either positive or negative. Therefore, if our hypotheses suggested a specific direction for group differences (e.g. more adverse childhood experiences in children from former indentured child laborers compared to children from a parental control group), Bayesian *t*-tests were tested accordingly.

For the BANCOVA's, the BF_{10} indicates the evidence for the full models (groups x parental rearing behavior variable) over the null model. Supplementary analyses (retrieve supplementary material from http://www.ejpt.net/index.php/ejpt/rt/suppFiles/30804/0) did not indicate that perceived rearing behavior from participating partners (i.e. parents that actively participated as former indentured child laborers or controls) differed from absent partners (i.e. parents we did not have data on, except the perceived rearing behavior scores from the respective offspring). Consequently, we aggregated maternal and paternal rearing behavior scores into family level variables by adding up scores of parental dyads. This was done under the assumption that one aspect of parental rearing behavior (i.e. maternal or paternal) cannot affect offspring psychopathology and wellbeing without interacting with the complementary aspect. Given that gender, financial status, and age of the participants were controlled for, these variables were included in the null model.

Our assumption was that children who described more family punishment, less emotionality and higher control indicated more psychopathology, a worse sense of coherence and less optimism/more pessimism. Furthermore, we mainly expected this pattern in families from former indentured child laborers. Therefore, we planned to analyze our date with nine separate analyses of covariance.

Results

Sample Characteristics

A total of *n* = 35 participants from the parental generation, including *n* = 16 former "Verdingkinder" and *n* = 19 parental controls with no history of childhood maltreatment, and *n* = 51 participants from the filial generation, including *n* = 22 adult children from former "Verdingkinder" and *n* = 29 adult children from parental controls, were enrolled in this study. The mean age of the both parental sub-sample was *M* = 74.31 (*SD* = 6.83) with *n* = 16 women (40.0%). The mean age of both filial sub-sample was *M* = 48.16 (*SD* = 8.09) with *n* = 35 women (68.7%). Table 5 shows the sample characteristics for the present analyses (see Table 5). Bayesian frequency analyses suggested that there was no evidence for a gender difference, marital status, or financial status in the parental samples. While we found no real support for an age difference, we found strong difference in years of education in the parental sub-samples (BF_{10} = 31.350). Regarding the filial sub-samples, the frequency analyses supported no gender difference, no difference in marital status or financial status. Regarding age, the difference was very strong (BF_{10} = 214.87), but there were no differences in years of education.

Table 5. *Sample characteristics of parental and filial data, divided by groups*

			ICL		Controls		
			M (SD) / n (%)		*M (SD) / n (%)*		BF_{10}
Parental Generation	*N*		16		19		
	Females		6	(37.50)	8	(42.11)	0.589
	Age		76.13	(6.81)	72.63	(5.96)	1.291
	Education		10.71	(1.65)	13.50	(2.99)	31.350
	Maritial status	Married	8	(50.00)	11	(57.89)	0.618
		Separated	1	(6.25)	3	(15.79)	
		Widowed	7	(43.75)	5	(26.32	
	Finace	Poor	1	(6.25)	0	(0.00)	0.785
		Fair or good	13	(81.25)	11	(57.90)	
		Very good	2	(12.5)	8	(42.11)	
Filial Generation	*N*		22		29		
	Females		15	(68.18)	20	(68.97)	0.458
	Age		52.91	(5.90)	44.55	(7.71)	> 100
	Education		13.91	(3.77)	15.55	(3.07)	0.933
	Maritial status	Single	3	(13.64)	9	(31.03)	0.531
		Married	12	(54.55)	15	(51.72)	
		Separated	7	(31.82)	5	(17.24)	
	Finace	Poor	2	(9.09)	0	(0.00)	1.089
		Fair or good	14	(64.64)	23	(79.31)	
		Very good	6	(27.27)	6	(20.69)	

Note. Independent sample *t*-tests and contingency tables were validated with a Bayesian approach (instead of null hypotheses significance testing). The here presented analyses were non-directional hypotheses in order to check if the two samples did not differ in demographic measures. ICL = Indentured child labor group.

Early-life Adversity and Trauma Across Generations

In a first step, we tested whether the parental samples differed with regard to early-life adversity. We found that the CTQ-SF group differences were strong for all dimensions (see Table 6), indicating meaningful differences in early-life adversity and trauma between parental samples. In a next step, we compared scores of the various subscales of the CTQ-SF in the offspring samples. Consistent with the parental findings, the offspring "Verdingkind" sample reported higher childhood trauma exposure than the offspring control sample (see Table 6). There were substantial differences between self-reported total CTQ-SF (BF_{10} = 2.476) and physical abuse (BF_{10} = 5.197), while emotional abuse (BF_{10} = 1.812), and emotional neglect (BF_{10} = 1.594) were only anecdotal, and those of physical neglect and sexual abuse yielded no support for our hypothesis (both $BFs_{10} < 1$).

Table 6. *Group differences on CTQ-SF scores across generations*

		ICL		Controls		
		M	*(SD)*	*M*	*(SD)*	BF_{10}
				Parental Generation		
CTQ-SF	**Total**	70.95	(22.18)	35.47	(7.25)	> 100
	Emotional abuse	12.61	(6.72)	6.63	(2.31)	66.21
	Physical abuse	13.31	(7.07)	6.16	(2.27)	> 100
	Sexual abuse	10.30	(6.94)	5.42	(1.02)	17.96
	Emotional neglect	20.00	(4.72)	9.90	(3.68)	> 100
	Physical neglect	14.38	(4.54)	7.37	(2.36)	> 100
				Filial Generation		
CTQ-SF	**Total**	40.23	(16.64)	33.34	(8.21)	2.476
	Emotional abuse	8.82	(3.09)	6.83	(5.04)	1.812
	Physical abuse	6.77	(3.78)	5.10	(0.41)	5.197
	Sexual abuse	5.86	(2.21)	5.62	(1.93)	0.392
	Emotional neglect	11.73	(5.85)	9.45	(3.99)	1.594
	Physical neglect	7.05	(3.05)	6.34	(1.84)	0.709

Note. Independent sample *t*-tests were validated with a Bayesian approach (instead of null hypotheses significance testing). It was assumed that indentured child labor (ICL) families indicated more childhood adversities and therefore, tested in a directional manner. CTQ-SF = childhood trauma questionnaire.

Parental Rearing Behavior, Psychopathology and Resilience in Offspring Samples

In a next step, we analyzed whether the offspring samples differed with regard to self-reported, recalled parental rearing behavior, psychopathology, optimism / pessimism and SOC. For the parental rearing behavior, scores were computed separately for maternal and paternal behavior. In line with our hypotheses, albeit only weakly for the paternal side, parental punishment has been found to be higher in the "Verdingkind" families (see Table 7). The "Verdingkind" offspring sample reported distinctly less emotionality by their fathers as compared to the control offspring sample ($BF_{10} = 14.246$). No difference was found in recalled paternal control and overprotection (both $BFs_{10} < 1.231$). Consistent with the paternal findings – but more pronounced – the same differences were found in maternal recalled rearing behavior. Controls reported to have perceived more emotionality by their mothers ($BF_{10} = 23.153$) and less punishment ($BF_{10} = 18.724$). As in the paternal control sample, there was no evident difference in maternal control between the two samples ($BF_{10} = 0.826$; see Table 7).

Table 7. *Group differences between the two filial samples on the QRPRB, the BSI, the SOC-R and the LOT-R*

		ICL		Controls		
		M	*(SD)*	*M*	*(SD)*	BF_{10}
QRPRB						
Father	Punishment	11.27	(4.13)	9.97	(2.13)	1.231
	Emotionality	17.73	(6.19)	22.26	(5.09)	14.246
	Control	12.52	(3.19)	12.91	(2.50)	0.418
Mother	Punishment	12.41	(4.32)	9.67	(1.74)	23.153
	Emotionality	20.05	(3.92)	23.93	(5.36)	18.725
	Control	14.50	(4.26)	13.34	(2.89)	0.826
BSI	**GSI**	18.41	(17.31)	15.97	(19.83)	0.408
SOC-R	**Total**	38.42	(6.01)	41.62	(5.70)	2.435
	Manageability	15.68	(3.34)	16.10	(2.58)	0.479
	Reflection	12.19	(2.28)	13.66	(2.11)	5.245
	Balance	10.55	(3.31)	11.86	(2.74)	1.386
LOT-R	**Total**	20.05	(4.91)	21.17	(4.46)	0.596
	Pessimism	4.32	(2.78)	3.03	(2.54)	1.741
	Optimism	9.21	(2.68)	9.36	(2.70)	0.246

Note. Independent sample *t*-tests were validated with a Bayesian approach (instead of null hypotheses significance testing). Indentured child labor (ICL) offspring were assumed to report more parental punishment, less emotionality and more control, more psychopathology and a lower sense of coherence. Furthermore, they were assumed to indicate more pessimism and less optimism. Hence, hypotheses were tested unidirectional. BSI GSI = brief symptom inventory global severity index; QRPRB = questionnaire of recalled parental rearing behavior; SOC-R = sense of coherence revised questionnaire; LOT-R = revised life orientation test.

We found no meaningful difference in psychopathology, as measured with the BSI-GSI (BF_{10} = 0.408), indicating no difference in mental health between offspring samples. Also, we found no evidence for a difference in optimism. However, "Verdingkinder" offspring reported anecdotally more pessimistic views than their controls (BF_{10} = 1.741). Finally, with regard to SOC, the total score of the SOC-R revealed an anecdotal difference between groups (BF_{10} = 2.560), indicating a stronger sense of coherence in the offspring control sample. This difference seemed to be mostly driven by the subscale 'reflection', as the higher scores of the offspring control sample were substantially different (BF_{10} = 5.245), while the difference in the 'balance' subscale was anecdotal (BF_{10} = 1.386), and the difference in 'manageability' was irrelevant in regard to our hypothesis (BF_{10} = 0.399; see Table 7).

Associations between Parental Rearing Behavior, Family Type and Mental Health of the Offspring Generation

With this step, we tested our exploratory hypothesis that parental rearing behavior depends on family type. As indicated above, maternal and paternal rearing behaviors for each participant were aggregated into three family level variables: family punishment, family emotionality and family control. To analyze whether family/group affiliation interacted with parental rearing behavior, a 2 (*indentured child labor family* vs. *control family*) x 1 (*parents rearing behavior*) Bayesian analysis of covariance (BANCOVAs) was conducted. The GSI score, SOC-R total and the LOT-R pessimism score were outcome variables in this analysis. All models were subsequently controlled for offspring's age and gender. QRPRB scores of paternal and maternal control did not differ between groups and

were, therefore, dropped for these analyses. This resulted in a total of six BANCOVAs (see Table 8).

Table 8. *Outputs of group x family emotionality and group x family punishment Bayesian analyses of covariance*

Group x Family Emotionality

Outcome Variables	*BSI GSI*	*SOC-R*	*Pessimism*
Group	0.647	2.398	0.377
Family Emotionality	16.839	0.393	23.151
Group * Family Emotionality	1.757	0.347	0.466
BF_{10}	**10.471**	**0.623**	**5.356**

Group x Family Punishment

Outcome Variables	*BSI GSI*	*SOC-R*	*Pessimism*
Group	0.26	3.22	0.365
Family Punishment	0.704	0.441	0.772
Group * Family Emotionality	0.207	1.01	0.392
BF_{10}	**0.133**	**1.636**	**0.278**

Note. Analyses of covariance were validated with a Bayesian approach (instead of null hypotheses significance testing). Bayes Factors (BF_{10}: in bolt) indicate the evidence for the full models (groups x parental rearing behavior variable) over the null model. Since gender, financial status, and age of the participants were controlled for, these variables were included in the null model. Non-bolt BF_{10} indicate analyses of effects within the tested model.

The models analyzing family emotionality where tested for main effects of group affiliation and family emotionality, as well as their interactions. The models revealed strong evidence for psychopathology ($BF_{10} = 10.471$), and substantial evidence for pessimism ($BF_{10} = 5.356$), indicating that psychopathology and pessimism are both affected by family emotionality and group affiliation. As can be seen in Figure 6, both of these results were determined by the main effect of family emotionality on psychopathology and family emotionality on pessimism (see Figure 6). Participants with lower family emotionality reported both, more psychopathology and pessimism. The main effect of group was insignificant in both models (both $BF_{10} < 0.647$). The interaction term of *group* x *family emotionality* appeared only anecdotal in the case of psychopathology ($BF_{10} = 1.757$), and was not evident in pessimism ($BF_{10} = 0.466$). The association with SOC appeared to be non-evidential ($BF_{10} = 0.623$), indicating that psychopathology might be partially dependent on family affiliation, while pessimism and SOC remained independent from it.

When focusing on family punishment and group affiliation, the BANCOVAs revealed only anecdotal evidence for an association with sense of coherence ($BF_{10} = 1.636$), but no evidence for associations with psychopathology or pessimism (both $BFs_{10} < 0.278$), indicating that family punishment and group affiliation did not affect psychological health of offspring in an evident manner.

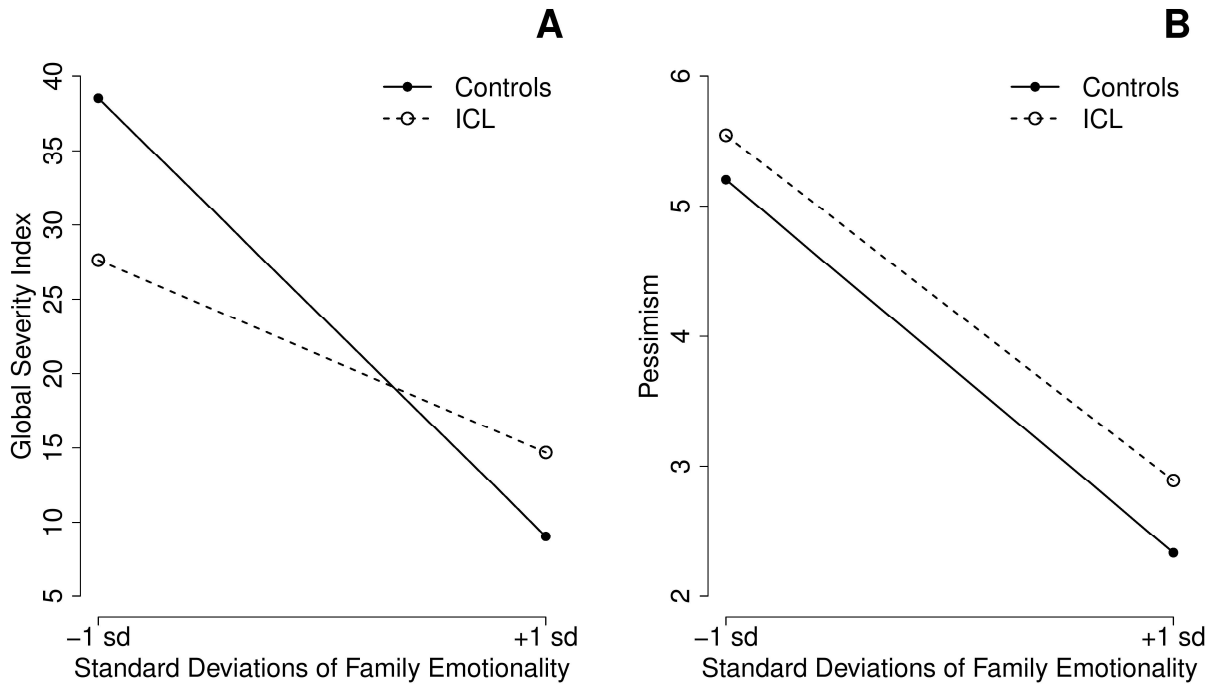

Figure 6. *Interaction plots for family emotionality and group affiliation on outcome variables.*

Note. Family emotionality is represented with ± *SD* from the mean **A.** Outcome variable is offspring psychopathology as indexed by the brief symptom inventory global severity index. **B.** Outcome variable is offspring pessimism, measured by the pessimism scale of the life orientation test.

Discussion

It was the aim of the current study to investigate the relationship between parental rearing behavior of former Swiss indentured child laborers, so-called "Verdingkinder" and psychological health of their adult offspring. We found that former "Verdingkinder" reported more adverse childhood experiences than controls. A similar pattern was observed in the "Verdingkinder" offspring sample, yet not as distinct as for the parental sample. What is more, while we found no meaningful group difference with regard to offspring's psychopathology, the "Verdingkinder" offspring sample reported substantially more physical abuse, and somewhat more emotional abuse and emotional neglect than their controls. Furthermore, the "Verdingkinder" offspring sample described their mothers and fathers as less emotional and described their mothers – and to a lesser extent their fathers – as more punitive. Although we found no differences in offspring's psychopathology, controls described themselves with a stronger sense of coherence, mainly due to more reflection and a less pessimistic view. Further exploratory analyses indicated that lower family emotionality was associated with more psychopathology and more pessimism, albeit mostly independent from group affiliation.

Our finding of higher levels of adversities in the parental sample of "Verdingkinder" was expected and in line with previous findings of our research group (Küffer et al., 2016). We did not find elevated levels of psychopathology in the "Verdingkinder" offspring sample when compared to offspring controls. This finding is consistent with results of a meta-analytic investigation studying secondary traumatization in the offspring of Holocaust survivors (Van IJzendoorn, Bakermans-Kranenburg, & Sagi-Schwartz, 2003), where the authors found no evidence for a transgenerational conveyance of parental trauma.

Nonetheless, although levels of psychopathology were not elevated, the "Verdingkinder" offspring sample reported more childhood adversities and trauma, indicating that the number of experienced adversities and trauma but not levels of psychopathology is higher in the offspring of trauma survivors.

Similar to the findings by Roth et al. (2014), our data do not support the notion of a "simple" transgenerational trauma transmission. In Roth et al. (2014), the authors did not find a direct association between maternal trauma or maternal PTSD and offspring psychopathology, and instead identified maternally experienced childhood violence (i.e. the violence the mother experienced during her childhood) as an important factor related to offspring psychopathology. The authors suggested that the familial context, such as maternal rearing behavior or parenting capacities, might have acted as a transmitting mechanism of maternally experienced early-life adverse experiences. Likewise, our results point in this direction, as in our sample, parental rearing behavior of former "Verdingkinder" has been described as more harmful by their offspring as compared to parental rearing behavior of controls. Unlike Field et al. (2013, 2011), who found that role-reversing parenting and parental overprotection were related to offspring's psychopathology (i.e. anxiety), we found that mainly maternal punishment and parental emotionality are key aspects of "Verdingkind" parental rearing behavior (while we did not assess role-reversing in our study). These differing findings may be explained by the fact that analyses by Field and colleagues were within-group analyses of Cambodian Khmer Rouge survivor offspring, while we compared two different Swiss samples. It is, therefore, possible that within trauma survivors, overprotection is the most important aspect of parental rearing behavior, but when focusing on differences between groups, punishment and emotionality are more relevant. While one expects within-group variance in a given

population, one does not necessarily expect differences in this dimension in our two samples, since the two groups shared most of their cultural frame of reference. The dimension of control and overprotection might be context- and culture-specific, and might be shared in the cultural context of our investigated groups.

We also found a weaker sense of coherence in the offspring of "Verdingkinder", which is in line with findings reported by Fossion et al. (2014), who found lower levels of resilience in adult offspring of less functional families. It is important to note here that we used a revised version of the original SOC questionnaire (i.e. SOC-R; Bachem and Maercker, 2016), while Fossion et al. (2014) applied the original construct by Antonovsky (1987). According to Bachem and Maercker, the SOC-R holds more distinctive variance, a better factor structure, and more stability over Antonovsky's (1987) SOC. Our finding adds, thus, to the growing body of literature that offspring of trauma survivors suffer not necessarily because of elevated psychopathology, but because of weakened resiliency capacities. But then again, one has to argue that although "Verdingkinder" offspring did experience more adversities and trauma, and experienced less than optimal parental rearing behavior, they did not develop elevated levels of psychopathology (as could have been expected), which might be considered a resilient aspect.

This study has a number of limitations: The sample sizes of both the parental and the offspring samples were rather small. Due to that, group comparisons allowed only for detection of larger effects, and most of the reported results – despite a trend in the assumed direction – remained anecdotal, or revealed to be statistically insignificant. In addition, our study was correlational, not allowing us to make causal conclusions. Therefore, our results should be replicated in a bigger sample, applying a prospective study design. Also, the selection of former child laborer families might not be representative with regard to family

functionality. For ethical reasons, participants of the parental generation were asked to establish contact with their children by themselves, and not by the research assistants. It is, therefore, possible and also confirmed by some of the former indentured child laborers that the relationship with their children was dysfunctional to the extent that they broke contact with their children altogether. As a consequence, our offspring sample might be a positive selection, implying that a more representative sample would have yielded more pronounced effects. Finally, our samples were not completely matched with regard to sample characteristics. The parental samples differed with regard to age and also reported substantially different levels of education. While the age difference may be considered rather small, the difference in years of education was fairly large. This can partially be explained by the fact that many former "Verdingkinder" were not given full access to education as compared to children with no "Verdingkind" background (Leuenberger & Seglias, 2008). It follows that a comparatively low level of education might be part of the demographic profile as a former child laborer. Future studies should match their samples with regard to these variables to exclude the possibility that our results were confounded by differences in demographic characteristics. The filial samples also meaningfully differed with regard to age. It might be speculated that, as a consequence of a lower level of education, former child laborers started their family earlier (Rindfuss, Morgan, & Offutt, 1996).

In conclusion, this is the first study that explored the transgenerational consequences of adverse childhood experiences in a sample of elderly former Swiss childhood laborers and their offspring. Future studies should include additional constructs from the positive spectrum of psychological health and functioning in order to approximate resilience more accurately.

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